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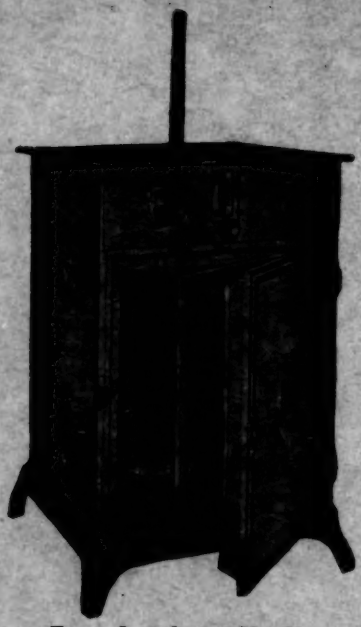

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CLINICAL AND EXPERIMENTAL

AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS

THE BLOOD PICTURE IN PURPURA*

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INTRODUCTION

THE complete blood examination is of paramount importance in the diagnosis, prognosis, and treatment of all cases showing purpura either as a predominant or secondary symptom. It is indeed surprising that (except in France) little attention has been paid to the blood changes until recently. The early reports of the diminution of the blood platelets in purpura by Brohm (1881), and E. Krauss (1883) in Germany, were ignored until Glanzmann[1] (1916) revived them. Denys[2] (1887) and Hayem[3] also reported similar observations on the blood platelets. Denys found a normal clotting time of the blood, and Hayem made the important observation of the absence of clot retraction and transudation of serum. Hayem[4] and his pupils (Bensaude, Rivet and others) deserve great credit for the preciseness of their studies of the main hematologic changes in purpura. Duke[5] noticed the prolonged bleeding time from the lobe of the ear and put this test on a working quantitative basis. Although this type of bleeding time may be prolonged in purpura, Leschke and Wittkower[6] point out that the operative bleeding time may not be unduly increased. Giffin and Holloway,[7] Leschke and older observers have seen patients with purpura stand major operations. Waltner[8] and Wittkower[9] have reported cases of uncomplicated labor in purpuric women. In both cases the newborns were also purpuric. Exsanguinating hemorrhages have followed tonsillectomy and childbirth in two cases in our series and required numerous transfusions to keep them alive. Artificial purpura can be brought about by the application of a tourniquet to the arm.

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This was first noticed by Grocco[10] and used later by Hess[11] as one of the main tests for purpura. This is called the tourniquet or capillary resistance test. In the Hess test the arm is compressed at 90 mm. of mercury for three minutes. Lewis[12] recently suggested the use of the sphygmomanometer at 70 mm. of mercury for three minutes, but this method shows a greater number of positive results than the ordinary rubber tourniquet which was used in the study of our cases. The greatest impetus in the study of purpura was given by Frank[13] when he published his theory concerning the myelotoxic influence of the spleen on the bone marrow. He introduced the term essential thrombopenia (diminution of the thrombocytes or blood platelets), which describes the main hematologic feature of purpura. Kaznelson[14] (1916) reported the first successful case of purpura treated by splenectomy; this was done as a result of his belief that the spleen was a destructive agent of the blood platelets producing thrombocytopenia. For that reason he used the name thrombolytic purpura.[15] Since then, numerous cases of purpura have been cured by splenectomy.

CLASSIFICATION OF PURPURA

The first classifications of purpura were mainly clinical (Henoch,[16] Litten[17]). It was thought that all purpuras were about the same, differing only in their intensity. Purpura which was limited to the skin was called purpura simplex, and where mucous membranes were also involved, it was called purpura hemorrhagica; purpura with additional joint involvements was called Schoenleins' disease; purpura associated with intestinal hemorrhages was called Henoch's purpura. Hayem,[4] Lenoble,[18] and Hutinel[19] have made classifications of the purpuras, according to the hematologic changes, which are more helpful. The first modern classification was made by Glanzmann,[1] who divided the purpuras into two groups: (1) anaphylactoid and (2) morbus maculosus Werlhofii. Recent classifications are those of Frank[20] and of Guglielmo[21]; the latter classification is as follows:

Guglielmo's Classification.

I. Morbus Maculosus Werlhofii.
   (b) Acquired Form, Acute or Chronic.
   (a) Constitutional Form, Chronic.

II. Schoenlein-Henoch's Purpura or Peliosis Rheumatica.

III. Symptomatic Purpura Hemorrhagica.
   (a) Diseases of the Blood (Myelemias, Aplastic, Pseudoaplastic, Hypoplastic, Hyperplastic, Aleukia, Hypoleukia, Splenic, etc.).
   (b) Endotheliosis.
   (c) Infectious Diseases, Avitaminosis, etc.

IV. Hemophilia.

V. Pseudohemophilia (Fibrinopenia).

The following classification is based on a clinical and hematologic study of 172 cases:

A. Purpura as a Result of the Dimunition of the Blood Platelets or Thrombocytopenic Purpura.
   1. (a) Acute Thrombocytopenic Purpura (deaths 6) (Table I).
      (b) Acute Thrombocytopenic Purpura (well 3) (Table I).

2. Chronic Thrombocytopenic Purpura, 47 cases.
   (a) Normal clot retraction, 4 cases (Table II).
   (b) Slight clot retraction, 11 cases (Table III).
   (c) No clot retraction, 32 cases (Tables IV, V, and VI).
3. Chronic Aplastic Anemia, 5 cases (Table VII).
4. Acute Aplastic Anemia, 5 cases (Table VII).

B. Symptomatic Thrombocytopenic Purpura.

1. Leucemia.
   (a) Leucopenic Myeloid, 18 cases (Table VIII).
   (b) Leucocytic Myeloid, 13 cases (Table IX).
   (c) Leucopenic Lymphatic, 6 cases (Table X).
   (d) Leucocytic Lymphatic, 4 cases (Table X).
2. Subacute Bacterial Endocarditis, 11 cases (Table XI).
3. Splenomegalic.
   (a) Resembling Banti's Disease, 8 cases (Table XII).
   (b) Gaucher's Disease, 7 cases (Table XIII).
   (c) Acquired Hemolytic Icterus, 1 case.
4. Pernicious Anemia, 5 cases (Table XIV).
5. Tuberculosis, 4 cases (Table XV).
6. Drugs.
   (a) Quinine, 1 case.
   (b) Salvarsan, 1 case.
7. (a) Carcinoma, 2 cases.
   (b) Dermoid Cyst of Ovary, 1 case.
8. Typhoid Fever, 1 case.

C. Chronic Thrombasthenic Purpura, 2 cases.
D. Hypertension and Nitrogen Retention, 6 cases (Table XVI).
E. Jaundice, 4 cases (Table XVII).
F. Avitaminosis (Scurvy) 3 cases (Table XVIII).
G. Schoenlein-Henoch's Purpura, 9 cases (Table XIX).
H. Purpura Fulminans, 1 case.

THE BLOOD PICTURE

The following blood examinations are the most important for the differentiation of the various types of purpura which we have studied:

1. Hemoglobin. This may be determined in percentage by the use of the new nonfade hemoglobinometer.

2. Red blood cells and white blood cells. Standardized pipettes and counting chamber should be used for the enumeration of the red and white cells.

3. Blood platelets. There are two chief methods of determining the number of blood platelets. First, 3 per cent sodium citrate freshly prepared is used as in performing the red cell count. The blood platelets are counted after they have settled in the counting chamber (Ottenberg and Rosenthal[22]). Second, by the use of citrated plasma, 0.03 c.c. of 30 per cent sodium citrate to each cubic centimeter of blood. The blood platelets are counted from the plasma after the red cells settle (Thomsen,[23] Gram[24]). The plasma is then diluted (1-20) with 3 per cent sodium citrate, and the platelets are enumerated in the ordinary counting chamber after sedimentation (fifteen minutes at least).

The morphology of the blood platelets should also be studied in smears or in citrated plasma. The blood platelets are usually very large in cases of thrombocytopenic purpura, pernicious anemia, and leucemia.

4. Differential count. This must be carefully done on preparations stained with the Jenner-Giemsa method (Rosenthal[25]). This method stains the abnormal cells extremely well for their proper identification (Rosenthal[26]).

5. Coagulation time of the blood. The Lee and White[27] method is the simplest and best. The blood is withdrawn from a vein for the coagulation time and for the platelet count at the same time.

6. Bleeding time. This must be done from the ear lobe.

7. Capillary resistance test. This can be done either with an ordinary rubber tourniquet or with the cuff of a sphygmomanometer, compressing the arm at 70 mm. of mercury, or at a pressure midway between systolic and diastolic, for three minutes. A positive test is shown by the appearance of petechiae after constriction.

8. Clot retraction. The blood used for the coagulation time is observed at half-hour or greater intervals. Normally, retraction begins within thirty minutes and is complete within four hours (Palmer[28]).

Blood changes were found in a great number of purpuric cases and were often diagnostic. Clinical symptoms alone are not sufficient for the determination of the exact nature of the underlying condition unless the case has already been diagnosed, such as typhoid fever or jaundice, and the purpura appears later as a complication. One of our patients with chronic thrombocytopenic purpura which later was cured by splenectomy, first entered the hospital with frank symptoms of Schoenlein-Henochs' disease (joint symptoms and hematuria). On account of the insufficient hematologic studies, the patient was discharged as such. During a later admission to the hospital, a complete blood study showed a low platelet count, prolonged bleeding time, positive tourniquet test, and absence of clot retraction, and the proper diagnosis was then made. It was not unusual for cases of purpura hemorrhagica in male children to be mistaken for hemophilia (pseudohemophilia). We have not seen true purpuric manifestations in our cases of hemophilia.

A. THE BLOOD CHANGES IN CASES OF PURPURA

1. *Acute Thrombocytopenic Purpura* (*Table I*).—The acute cases of purpura differ from cases of chronic purpura only in their course. There are three varieties when first seen: (1) they may run a fatal course, as in six cases reported; (2) they may recover spontaneously (apparently the first case of purpura reported by Werlhof belonged to this group), and (3) the case may be the onset of a chronic type. The blood pictures of all the acute cases are characterized by a marked diminution of the blood platelets. The diminution of the hemoglobin and red blood cells depends upon the amount of hemorrhage which has taken place. When first seen, the hemoglobin and red blood cells may be normal, but after excessive blood loss, one finds a proportionate fall in the hemoglobin and red blood cells. Case R.F., Table I, had rather high hemoglobin and red blood cells on admission to the hospital, but within two weeks, the hemoglobin and red blood cells fell to very low figures. A citrate

TABLE I

ACUTE THROMBOCYTOPENIC PURPURA

NO.	NAME	HGB.	RBC.	WBC.	PL.	PN.	L.	M.	CT.	BT.	TT.	CR.
1	R.F.	90	4,8	8	1	54	38	5	8	25	+	None
2	C.M.	88	4,3	12	1	73	21	5	19	19	+	None
3	H.E.	22	1,1	7	2	62	23	11	14	120	+	None
4	S.W.	48	3,6	12	5	80	18	2	6	80	+	None
5	A.T.	85	5,0	6	10	70	22	3	12	6	+	Slight
6	I.G.	24	1,6	8	17	79	16	5	8	190	+	Slight
7	M.M.	42	2,3	16	20	73	20	3	11	8	+	None
8	R.G.	64	3,5	12	20	59	37	4	6	60	+	None
9	P.D.	84	4,4	7	20	60	32	8	9	19	+	None

The following abbreviations are used for the tables:

HGB.—Hemoglobin. RBC.—Red Blood Cells (in millions). WBC.—White Blood Cells (in thousands). PL.—Platelets (in thousands). PN.—Polynuclear Neutrophils. L.—Lymphocytes. M.—Monocytes. CT.—Coagulation Time. BT.—Bleeding Time. TT.—Tourniquet Test. CR.—Clot Retraction.

transfusion given to this patient was followed by an increased amount of bleeding. This was attributed to the citrate, but the same excessive bleeding occurred after a direct transfusion.

The white blood cells are either normal in number or somewhat increased. The differential blood picture is about normal, except in two cases where the monocytes were increased to 8 and 11 per cent. The coagulation time is normal or somewhat prolonged in all cases. The special tests show the characteristic changes as a result of the diminution of the blood platelets, the normal or increased coagulation time, greatly increased bleeding time, positive tourniquet test, and failure of the proper retraction of the blood clot in all cases. The blood picture in the acute cases is similar to that of the chronic cases.

2. *Chronic Thrombocytopenic Purpura* (*Tables II, III and IV*).—The cases of chronic thrombocytopenic purpura are the most numerous. The outstanding symptoms of all the cases are the hemorrhages into the skin and from the mucous membranes. Some of the cases reported here have been followed for years. The blood platelets returned to normal, and the purpura and hemorrhagic tendency disappeared in a few cases at times (intermittent purpura). As a rule the diminution of the platelets is always present. The purpuric condition in some cases is very latent and runs a mild course.

All the manifestations of this disease can be said to be due to the diminution of the blood platelets in the circulating blood. This diminution in the blood platelets is believed to be due either to a disease of the bone marrow, most likely of the megakaryocytes (the mother cells of the blood platelets), or to an increased destruction in the spleen. Frank believes in a myelotoxic influence of the spleen affecting the formation of the platelets. Jedlicka and Altschuller[29] find distinct morphologic changes in the megakaryocytes of the bone marrow stained with Giemsa. On the other hand, Kaznelson[15] believes that there is an increased destruction of blood platelets in the spleen to account for the thrombocytopenia. This has been the *raison d' être* for the cure or treatment of this disease by splenectomy. In the study of 22 postoperative cases of this type of purpura treated by splenectomy, I[30] have come to the conclusion that both ideas are correct. In some cases, the thrombocytopenia is due to a diminished formation of blood platelets in the

bone marrow, and in other cases, there is an increased destruction. In the first group, the blood platelets, after preliminary increase, return to their former low level, and in the second group, the blood platelets, after a much greater preliminary increase, return to normal figures. We may consider the splenic factor as an aggravating one in the first condition, and as a primary disease of the spleen in the second group.

The blood platelets not only vary in number in this disease but also in their quality. The most frequent blood picture encountered in this group is a normal hemoglobin and red blood cell count (or diminution comparable to the amount of hemorrhage), a normal white cell count, and a normal differential count. Leucocytosis (polynucleosis and monocytosis) may occur after hemorrhage. The blood platelets vary from 2,000 to 100,000, the average

TABLE II

CHRONIC THROMBOCYTOPENIC PURPURA SHOWING PRESENCE OF CLOT RETRACTION

NO.	NAME	HGB.	RBC.	WBC.	PL.	PN.	L.	M.	CT.	BT.	TT.	CR.
1	S.G.	62	3,4	7	5	66	29	5	6	12	–	Present
2	E.B.	69	4,7	6	48	62	27	7	8	3	+	Present
3	L.M.	76	5,3	10	50	70	28	2	7	3	+	Present
4	E.P.	98	4,8	13	80	57	40	3	12	2	+	Present

TABLE III

CHRONIC THROMBOCYTOPENIC PURPURA SHOWING SLIGHT CLOT RETRACTION

NO.	NAME	HGB.	RBC.	WBC.	PL.	PN.	L.	M.	CT.	BT.	TT.	CR.
1	M.L.	84	5,1	17	5	58	35	6	6	17	+	Slight
2	E.H.	77	3,8	11	10	59	38	3	10	8	+	Slight
3	M.B.	85	4,8	11	10	68	26	5	7	18	+	Slight
4	M.A.	67	3,4	10	20	64	31	3	5	10	+	Slight
5	L.C.	80	5,3	10	20	54	40	4	6	6	+	Slight
6	F.D.	70	5,1	9	20	68	17	14	11	30	+	Slight
7	J.G.	91	5,3	7	30	67	24	7	10	9	+	Slight
8	M.M.	114	7,5	11	30	65	22	7	5	9	+	Slight
9	A.B.	36	1,8	4	40	66	25	8	9	8	+	Slight
10	R.A.	60	3,3	5	50	68	27	4	16	5	–	Slight
11	M.H.	30	1,9	5	100	37	60	2	6	5	+	Slight

TABLE IV

CHRONIC THROMBOCYTOPENIC PURPURA SHOWING ABSENCE OF CLOT RETRACTION AND PLATELETS BELOW 10,000

NO.	NAME	HGB.	RBC.	WBC.	PL.	PN.	L.	M.	CT.	BT.	TT.	CR.
1	F.F.	66	5,4	8	2	60	30	8	8	26	+	None
2	B.S.	70	3,5	11	2	59	34	7	6	6	+	None
3	E.G.	50	2,8	6	4	84	13	3	11	10	+	None
4	M.N.	58	3,5	8	4	76	22		13	6	+	None
5	A.G.	98	5,6	6	5	80	12	8	12	16	+	None
6	M.H.	40	2	8	5	63	32	4	12	15	+	None
7	M.S.	70	3,8	20	5	71	13	8	7	10	+	None
8	S.H.	92	4,8	11	6	64	20	3	6	6	+	None
9	R.G.	38	2,2	6	6	54	26	13	10	10	+	None
10	L.C.	74	3,9	8	8	67	27	4	9	25	+	None
11	J.W.	91	4,8	8	8	71	21	6	13	21	+	None
12	E.G.	94	5,1	12	10	65	31	5	10	42	+	None
13	Y.K.	86	4,2	10	10	72	20	6	4	16	+	None
14	B.L.	79	4,8	12	10	73	19	6	6	10	+	None
15	J.L.	80	5,7	10	10	45	53	2	6	13	+	None
16	S.R.	96	4,8	13	10	76	15	9	8	9	+	None

TABLE V

CHRONIC THROMBOCYTOPENIC PURPURA SHOWING ABSENCE OF CLOT RETRACTION AND PLATELETS ABOVE 10,000

NO.	NAME	HGB.	RBC.	WBC.	PL.	PN.	L.	M.	CT.	BT.	TT.	CR.
1	D.R.	64	3,1	8	20	64	37	8	8	8	+	None
2	R.L.	80	4,2	13	34	57	41	2	8	4	+	None
3	R.B.	75	4,5	11	25	72	21	3	10	10	+	None
4	M.A.	98	5,1	12	30	73	17	7	6	17	+	None
5	G.L.	98	4,6	10	30	52	42	4	8	10	+	None
6	J.Y.	35	1,8	18	30	78	18	3	7	16	+	None
7	M.O.	40	2,3	15	40	91	4	5	20	8	+	None
8	C.S.	37	3,2	4	42	48	33	11	13	20	+	None
9	R.J.	39	1,9	8	45	77	19	3	9	6	+	None
10	J.C.	83	5,0	14	50	68	27	3	6	6	−	None
11	H.S.	84	4,3	14	50	79	15	6	10	16	+	None
12	S.R.	70	3,9	23	80	83	13	4	5	7	+	None
13	C.G.	63	3,2	21	90	71	24	3	8	20	+	None

TABLE VI

CHRONIC THROMBOCYTOPENIC PURPURA WITH NORMAL BLEEDING TIME

NO.	NAME	HGB.	RBC.	WBC.	PL.	PN.	L.	M.	CT.	BT.	TT.	CR.
1	C.T.	125	5,5	12	5	71	23	6	11	1	+	None
2	B.T.	75	4,1	5	5	45	52	2	12	2	+	None
3	H.H.	84	4,3	9	6	76	18	3	7	2	+	None

platelet count being 25,000. The bleeding time does not seem to be associated with the number of platelets. In three cases the bleeding time was normal (Table VI), although the blood platelet average was 5,500. The coagulation time is normal or slightly prolonged. The tourniquet test was preesnt in all cases with the exception of two. The clot retraction was absent or slight in all cases except in four, in which the clot retracted normally. (Table II.) In one of these cases, a splenectomy was performed with excellent results; the patient later had a subtotal gastrectomy done without any hemorrhage.

We can deduce from the study of all these cases that the cause of the hemorrhagic phenomena is due not only to the diminution in the blood platelets, but also to some change in their quality. Their altered function can be determined by the special tests outlined; mainly the coagulation time, bleeding time, tourniquet test, and clot retraction, which correspond to the following functions of the blood platelets:

1. Coagulative function. It is beyond the scope of this paper to take up the various theories concerning the coagulation of the blood. The important relation of the blood platelets in instituting the coagulation of the blood has long been pointed out by Hayem,[31] Salvioli,[32] Bordet and Delange,[33] and also recently by Tait.[34] This factor is apparently not involved in cases of purpura. It has been pointed out, however, that serum obtained from a blood clot poor in platelets contains less thrombin as measured by its action on fibrinogen or hydrocele fluid.

2. Hemostatic function. Hayem[31] has pointed out the vulnerability, the viscousness, and the agglutination of platelets to foreign bodies. Wright and Minot[35] have observed and produced the viscous metamorphosis of the platelets. Hayem had also noticed the agglutinative power of blood platelets,

and their adhesions to wounds which he terms the "clou hemostatique," the method by which hemorrhage is prevented from the capillaries. In purpura hemorrhagica this property is greatly interfered with on account of the blood platelet diminution and some change in their quality. In other diseases, such as pernicious anemia and leucemia, there is also a diminution of the blood platelets without change of quality, as a rule, so that the bleeding time may be normal, and purpuric manifestations may not occur. In purpura, however, an increase in blood platelets, even up to 100,000 and in a few cases higher still, may be accompanied by a prolonged bleeding time. The bleeding time was normal in only three cases (Table VI).

3. Tourniquet Test. The tourniquet test indicates the condition of the capillaries. The appearance of petechiae at the elbow after the application of the tourniquet is not pathognomonic of purpura. It occurs in a great many other conditions. There is a difference in the results of this test in the use of an ordinary rubber tourniquet and the broad cuff of a sphygmomanometer. The latter produces positive results in a greater number of diseases than the former. Hyperthyroidism, hypertension, chronic nephritis, pernicious anemia, subacute bacterial endocarditis, and leucemia uniformly show positive reactions by the use of the sphygmomanometer. In some of these cases the test may be negative by the use of the ordinary rubber tourniquet. By the use of the rubber tourniquet, practically all the cases of purpura show a positive reaction.

The relation of the blood platelets to the capillaries is very important. Frank believes that the platelets prevent the diapedesis of red blood cells in the normal individual by stopping the various stomata between the capillaries which occur from the traumas of daily life. The diminution of the number of blood platelets in purpura interferes with this function because of the additional qualitative change. In other diseases where the blood platelets may be just as low as in thrombocytopenic purpura, purpuric manifestations are not very common as one can see from the number of cases reported in the various tables; a great many cases of pernicious anemia and subacute endocarditis with marked diminution of the blood platelets have not been included in this communication on account of the absence of purpura. Leschke and others believe that there is some alteration of the capillaries in thrombocytopenic purpura. In a few cases of purpura which we have followed, purpuric skin manifestations rarely occur but are easily produced by slight traumas. The so-called spontaneous purpuric manifestations are probably due to unnoticed or very slight traumatic changes of the capillaries. Even changes in venous blood pressure may bring on attacks of apparently spontaneous purpura, as one notices frequently in the legs (orthostatic purpura). The latter form of purpura may also occur in less well-known diseases of the capillaries which show increased fragility without any evidence of changes in the blood platelets (Arnett and Weidman[36]).

4. Clot Retraction. Hayem was the first one to point out the absence of clot retraction in cases of purpura during the active stage of the disease. His coworkers have also found this to be present in symptomatic purpuras where a diminution of blood platelets is also found. The experiments of

TABLE VII

ACUTE AND CHRONIC APLASTIC ANEMIA

NO.	NAME	HGB.	RBC.	WBC.	PL.	PN.	L.	M.	CT.	BT.	TT.	CR.
1	M.M.	33	1,1	1	1	33	58	5	15	27	+	None
2	R.A.	15	8	1	1	19	68	5	14	20	+	None
3	J.S.	24	7	1	1	10	89	1	15	22	+	None
4	M.L.	42	3,2	2	2	45	49	4	9	20	+	Slight
5	A.S.	40	1,7	1	30	22	72	5	6	8	+	Slight
6	A.S.	51	2,5	3	2	32	58	10	7	6	+	None
7	J.T.	25	1,4	2	5	29	63	7	16	10	+	None
8	A.W.	44	1,8	2	20	32	64	2	6	45	+	Slight
9	H.O.	29	1,5	1	20	44	46	7	15	13	+	None
10	J.G.	49	2,4	3	10	40	42	2	8	8	+	None

Cases 1-5 Acute Aplastic Anemia.
Cases 6-10 Chronic Aplastic Anemia.

Le Sourd and Pagniez[37] have definitely proved the relation of the blood platelets to clot retraction. Arthus and Chapiro[38] have shown that the clot retraction depends upon the integrity of the blood platelets. Sodium fluoride, distilled water, and cold which injure the blood platelets retard retraction. Sodium citrate and sodium oxalate which conserve blood platelets do not hinder clot retraction. It is exceptional to find clot retraction in cases of purpura hemorrhagica; this was observed only in four cases where the blood platelets varied from 5,000 to 48,000.

There can also be a dissociation of function of the blood platelets. (1) The blood platelets may retain the adhesive properties and retractive properties, and cause the purpuric phenomena solely through their diminution in number. (2) The blood platelets may possess the adhesive properties but may be deficient in the retractive property. (3) The platelets may show both quantitative and qualitative changes.

The blood studies confirm Hayem's idea[4] that the two most important blood changes in true purpura are the diminution of the blood platelets and the loss of clot retraction.

3. *Chronic Aplastic Anemia* (*Table VII*).—Chronic aplastic anemia is separated from the chronic purpuric cases on account of the difference in their course and the blood picture. They resemble pernicious anemia on the one hand, except that there is an absence of macrocytosis, and thrombocytopenic purpura on the other hand, except that the white blood cells are persistently low, with a relative lymphocytosis. We have had occasion to study five cases. Splenectomy was tried unsuccessfully in three; two died within a month after the operation, and one patient is still living but has a very severe anemia. They all had attacks of purpura with the characteristic alterations in functions of the blood platelets. In one case, macrophages were present and a positive blood culture of Streptococcus anhemolyticus was later reported.

4. *Acute Aplastic Anemia* (*Table VII*).—These cases were of short duration. They were all characterized by the presence of marked hemorrhagic phenomena. In Case 4, the bleeding had to be stopped at the end of twenty minutes by compression of the ear with gauze. Twenty-four hours later, when the gauze was removed, bleeding began again. The blood picture is charac-

terized by a marked diminution of all the formed elements of the blood, and a relative lymphocytosis. The bleeding time, capillary resistance test, and clot retraction were the same as in thrombocytopenic purpura.

B. SYMPTOMATIC THROMBOCYTOPENIC PURPURA

Certain diseases such as leucemia, subacute endocarditis, and pernicious anemia may be associated with purpura. As a rule the underlying condition can be determined by clinical and laboratory examinations. The true nature of the purpura cannot be determined without the complete blood picture.

1. *Leucemia (Tables VIII, IX, and X).*—Leucemias with a normal or diminished white cell count simulate thrombocytopenic purpura very closely. The symptoms may be identical. The only means of differentiating the two conditions is the blood picture. As a rule the anemia is out of proportion to the amount of blood lost, but otherwise the platelets and the special tests

TABLE VIII

LEUCOPENIC MYELOID LEUCEMIA

NO.	NAME	HGB.	RBC.	WBC.	PL.	PN.	L.	M.	MCN.	MBST.	CT.	BT.	TT	CR.
1	H.P.	44	2,0	1	38	18	61	6	1	14				
2	J.G.	50	2,6	2	6	6	52	2	4	35	5	20	+	None
3	R.S.	24	1,5	2	2	16	37			47	12	24	+	None
4*	S.T.	42	2,2	3	40	27	29	44			10	8	+	None
5	I.R.	27	1,1	3	36	13	65	1	1	19	7	3	+	None
6	H.Z.	30	1,0	3	60	42	40	8	4	47	10	4	+	Slight
7	E.B.	18	1,4	5	60	40	41	8	7	3	6	3	+	Normal
8	J.H.	80	4,0	5	9	18	65		4	13	12	24	+	None
9	M.W.	58	2,7	5	10	17	71	2	2	8	14	15	+	None
10	A.W.	14	8	8	20	44	18		16	22				
11	S.S.	30	1,4	9	35	61	11	1	8	19	10	16	–	Slight
12	S.R.	38	1,9	9	6	50	39	4	4	3	7	4	+	Slight
13	M.C.	44	2,4	11	32	5	8			87	5	20	+	None
14†	S.W.	52	2,2	12	30	68	18	1	10	3	4	5	–	None
15	T.F.	30	1,6	13	96	68	4	2	6	20	10	2	+	Normal
16	D.S.	30	1,6	13	20	31	45	2	8	11	12	6	+	None
17	L.D.	69	4,4	18	40	4	25	3	5	63	24	1	–	None
18	B.W.	56	2,9	20	4	26	27		1	70	7	3	+	Slight

*Monocytic leucemia.
†Splenectomy—death.
MCN.—Myelocytes Neutrophilic
MBST.—Myeloblasts

TABLE IX

MYELOID LEUCEMIA

NO.	NAME	HGB.	RBC.	WBC.	PL.	PN.	L.	M.	MCN.	MBST.	CT.	BT.	TT	CR.
1	M.C.	20	1,3	28	15	13				87	4	3	+	Normal
2	H.W.	19	1,0	30		1	1	2	10	85	12	120	+	None
3	B.M.	42	1,5	32	20	4	10	1		85	4	30	+	Normal
4	B.B.	33	1,6	47	10	2	14			85	8	20	+	None
5	A.G.	13	7	53	20	5	4	1	1	88	4	20	+	None
6	A.H.	40	1,8	63	30	5				95	7	13	+	None
7	A.P.	58	2,8	71	50		2			97	10	15	+	None
8	G.F.	62	3,8	100	50	41	40	6	7	5	14	15	+	None
9	C.I.	50	2,9	124	15	20	26	14	9	21	5	6	+	None
10	B.M.	30	2,6	135	15	20	10			54	15	25	+	None
11	D.G.	93	4,6	141	60	2	20			78	11	30	+	None
12	I.K.	32	1,5	200	55	20	8		2	88	3	12	+	Slight
13	M.K.	30	1,2	600	20				1	97	10	8	–	None

MCN.—Myelocytes Neutrophilic
MBST.—Myeloblasts

TABLE X

LYMPHATIC LEUCEMIA

NO.	NAME	HGB.	RBC.	WBC.	PL.	PN.	L.	M.	LBST.	CT.	BT.	TT.	CR.
1	J.S.	30	3	5	85	1	99			2	14	+	Normal
2	I.F.	32	1,7	7	12	1	57	1	40	8	10	+	None
3	H.C.	120	5,9	8	120	26	74			9	5	+	Slight
4	S.F.	80	4,3	8	50	20	77	3		10	4	+	Slight
5	H.E.	52	4,1	13	60	17	83	1		10	8	–	Normal
6	J.S.	101	5,3	23	1	13	83			12	20	+	None
7	L.B.	83	5,1	33	25	15	81			4	1	–	Normal
8	A.M.	49	1,5	41	10	2	16		81	14	1	+	None
9	T.B.	35	1,8	42	6	2	64	4		11	120	+	None
10	S.G.	45	2,2	69	50	2	98			4	10	+	Slight

LBST.—Lymphablasts

TABLE XI

SUBACUTE BACTERIAL ENDOCARDITIS WITH PURPURA

NO.	NAME	HGB.	RBC.	WBC.	PL.	PN.	L.	M.	MAC.	CT.	BT.	TT.	CR.
1	A.S.	59	3,3	13	8	95	3	1		11	6	+	None
2	M.D.	54	3,1	2	18	69	24	5		9	5	+	None
3	B.C.	70	3,4	4	35	59	31	10		8	5	+	Slight
4	A.F.	67	3,6	5	50	65	26	8	1	8	4	+	None
5	E.E.	74	3,6	5	70	70	28	2		7	2	–	Slight
6	R.G.	49	2,5	7	90	71	22	6		9	3	–	Normal
7	H.K.	75	4,7	42	5	65	20	4	10	12	10	+	Slight
8	D.V.	58	2,7	5	130	62	31	6		4	2	+	Normal
9	J.G.	70	4,5	5	130	27	50	6	12	4	3	+	Normal
10	A.F.	72	4,2	5	160	79	18	3		6	2	+	None
11	S.E.	50	3,9	4	200	60	26	5	3	7	1	–	Normal

MAC.—Macrophages

correspond to those found in thrombocytopenic purpura. The important differential point is the presence of premature cells, such as neutrophilic myelocytes, myeloblasts and their forerunners, the myelogones, in the leucopenic myeloid leucemias, and the lymphocytosis and lymphoblasts in the leucopenic lymphatic leucemias. The differential blood count is therefore extremely important in all cases of purpura. The staining methods and the differentiation of the various premature white blood cells have been discussed in a previous communication. The leucocytic leucemias offer no difficulty in diagnosis. The high white cell count is diagnostic. It is interesting to note that most of the leucemias which are associated with purpura are of the myeloblastic type.

2. *Subacute Bacterial Endocarditis* (*Table XI*).—In a large series of cases (over 100) studied as outlined above, the blood picture was quite varied. The secondary anemia was progressive in practically all of the cases. The leucocytes varied from a marked leucopenia (usually in the bacterial free stage with splenomegaly) to leucocytosis. The platelets also show the same variations. The differential blood picture showed marked changes, polynucleosis in some, lymphocytosis in others, and in about 10 per cent of the cases, macrophages or histocytes were found in the blood smear from the ear. Skin manifestations were present in practically all, consisting of bright red petechiae, white centered petechiae, and in about twenty patients purpura was present. The blood of eleven of these patients was carefully studied. In five, macrophages were present at one time or another. In most of the cases the platelets were very low, and in two cases the platelets were 160,000 and 200,000, with normal bleeding time in both. The tourniquet test was positive in eight of

TABLE XII

SPLENOMEGALY AND PURPURA SHOWING TENDENCY OF DIMINUTION OF WHITE CELLS AND PLATELETS, RESEMBLING CASES OF BANTI'S DISEASE

NO.	NAME	HGB.	RBC.	WBC.	PL.	PN.	L.	M.	CT.	BT.	TT.	CR.
1	M.H.	28	1,6	2	0	83	15	2	8	7	+	None
2	F.S.	52	3,3	5	10	64	27	8	10	6	+	None
3	A.I.	20	1,6	3	15	57	40	3	6	9	+	Slight
4	E.B.	32	2,4	2	20	73	18	8	8	9	+	None
5	J.S.	100	4,4	3	40	54	38	6	10	4	−	Slight
6	E.L.	46	2,3	3	60	39	60	1	12	8	−	None
7	J.D.	26	1,5	4	70	80	16	4	5	2	−	Normal
8	A.S.	88	4,9	9	145	58	38	4	8	3	+	Normal

TABLE XIII

GAUCHER'S DISEASE

NO.	NAME	HGB.	RBC.	WBC.	PL.	PN.	L.	M.	CT.	BT.	TT.	CR.
1	F.R.	55	3,1	3	30	47	50	3	5	9	+	Normal
2	H.L.	57	2,8	1	32	67	23	6	15	6	+	None
3	L.T.	53	3,3	4	50	57	39	4	7	7	+	Slight
4	I.R.	90	4,6	5	60	73	22	4	10	2	+	Slight
5	S.P.	90	5,0	2	60	74	21	4	10	6	+	Slight
6	S.F.	60	3,6	2	90	46	52	2	11	5	+	Slight
7	M.C.	45	3,1	2	110	52	38	8	7	3	+	Normal

the eleven cases. In three cases there was an absence of clot retraction. In the others the clot retraction was either normal or slight. The purpuric changes present in subacute bacterial endocarditis can be said to be due to some capillary change as well as some alteration of the function of the blood platelets. The latter is not as pronounced as it is in true cases of purpura. Clinical phenomena are more important in the diagnosis of this disease, although the finding of macrophages in certain obscure cases without a positive blood culture makes the diagnosis of this condition certain.

3. *Splenomegalic.*—

(a) In eight patients with splenomegaly whose blood pictures resembled that found in Banti's disease, purpuric manifestations were very marked and, except for the leucopenia, may be classified as thrombocytopenic purpura (Cases 1, 2, 3, 4, and 7). All these patients reacted favorably after splenectomy. In the other three cases, purpura was very slight and transitory, and did not play an important rôle. Case 5 was Banti's disease in the third stage; splenectomy was followed by death. The relation of the blood platelets to Banti's disease is a very interesting one and has been reported previously. (Rosenthal.[39]) (Table XII.)

(b) Gaucher's Disease (Table XIII). Seven patients with Gaucher's disease showed the presence of a typical purpura; the characteristic diminution of the blood platelets, slightly increased bleeding time, positive tourniquet test, and normal clot retraction were found in two cases, and absence of the clot retraction in the others. The diagnosis of this condition cannot depend upon the blood examination alone. The clinical manifestations of the disease, enlargement of both liver and spleen, pigmentation, pingueculae, and sometimes gross or x-ray bone changes are necessary for the diagnosis. When the liver and spleen alone are enlarged, without the other changes, bone or splenic puncture may be required for the diagnosis of the condition.

TABLE XIV

PERNICIOUS ANEMIA

NO.	NAME	HGB.	RBC.	WBC.	PL.	PN.	L.	M.	CT.	BT.	TT.	CR.
1	S.I.	33	1,4	2	20	51	46	3	9	8	–	Slight
2	M.M.	40	1,5	5	50	54	41	2	9	8	–	Normal
3	M.R.	76	3,4	4	80	37	61	2	10	3	+	Normal
4	A.S.	44	1,4	4	80	58	35	3	9	5	+	Slight
5	M.Y.	34	1,1	4	90	59	34	3	8	7	+	Slight

TABLE XV

TUBERCULOSIS

NO.	NAME	HGB.	RBC.	WBC.	PL.	PN.	L.	M.	CT.	BT.	TT.	CR.
1	M.C.	90	4,7	6	2	68	27	6	12	17	+	None
2	P.L.	33	2,7	12	25	72	19	8	5	16	+	None
3	J.S.	64	4,0	14	64	37	43	14	10	8	+	None
4	D.O.	40	2,6	9	80	79	17	4	12	19	+	None

(c) Acquired Hemolytic Icterus. Purpura was found in one case of acquired hemolytic icterus. The blood platelets were diminished to 80,000, but these were apparently of good quality, as the bleeding time and clot retraction were normal and the tourniquet test was negative. Bile in the blood and increased fragility of the red cells were present. Hemoglobin, 32 per cent; red blood cells, 2,600,000; white blood cells, 3,000; platelets, 80,000; polymorphonuclear neutrophils, 76 per cent; lymphocytes, 20 per cent; monocytes, 4 per cent; coagulation time, four minutes; bleeding time, three minutes; tourniquet test, negative; clot retraction, normal.

4. *Pernicious Anemia* (*Table XIV*).—The blood picture is the most important means of making the diagnosis of pernicious anemia. The high color index and the macrocytosis is rarely found in other conditions, except occasionally in leucopenic leucemia. The differential blood count in pernicious anemia shows a slight lymphocytosis and rarely the presence of premature blood cells. Normoblasts and megaloblasts are rather infrequent in pernicious anemia, and, as a rule, are present in very large numbers in the leucopenic leucemias. Most of the cases showed a marked diminution in the number of blood platelets. Purpura is not common and was found in only five cases. The qualitative change in the blood platelets is not as great as it is in purpura.

5. *Tuberculosis* (*Table XV*).—Purpura is rather uncommon in tuberculosis. In two of the cases the purpuric manifestations were the outstanding features. The blood picture was typical of thrombocytopenic purpura in both. Further observations of the cases disclosed the presence of tuberculosis. Case 1 (Table XV) revealed the presence of tuberculosis in an excised lymph node, and the x-ray examination of the chest showed the presence of miliary tuberculosis. In Case 2 tuberculosis was found in one kidney, which was excised. The purpura disappeared in both kidneys, and the blood picture in Case 2 became normal; the blood platelets increased in number, and the special tests for their quality also showed normal results. Case 3 was a child who showed clinically a severe anemia, marked enlargement of the spleen, and purpura. Examination of the spleen after splenectomy showed microscopic tuberculosis. For three years after the operation the child showed a blood picture which resem-

bled monocytic leucemia and absence of a hemorrhagic tendency. Case 4 showed a terminal purpura in general miliary tuberculosis.

6. *Purpura as a Result of the Intake of Drugs.*—It is usually stated that numerous drugs may produce purpura. Skin manifestations nonpurpuric in character are frequent as a result of the intake of various drugs. True purpura as a result of drugs was found in only two cases. One patient had an idiosynerasy for quinine and would always develop a purpuric eruption following its ingestion. The blood was examined during one attack and was found that the blood platelets were markedly diminished, coagulation time was normal, bleeding time was normal, tourniquet test became strongly positive, and no clot retraction was present. The purpura disappeared within a week, and the idiosynerasy was tested by injecting a minute quantity of urea and quinine hydrochloride intradermally. As a result he developed a profuse purpura scattered all over the body. Hemoglobin, 90 per cent; red blood cells, 5,000,000; white blood cells, 16,000; platelets, 90,000; polymorphonuclear neutrophils, 50 per cent; lymphocytes, 42 per cent; monocytes, 6 per cent; coagulation time, nine minutes; bleeding time, three minutes; tourniquet test, positive; clot retraction, none.

Purpura after salvarsan was observed in one case, although this was stated to be more frequently found in France where the subject has been thoroughly investigated (Mattas[40]). In the case under observation, the purpuric manifestations cleared up after three citrate transfusions, with the restoration of a normal blood picture. The blood picture in this case showed typical findings of thrombocytopenic purpura. Hemoglobin, 42 per cent; red blood cells, 2,900,000; white blood cells, 14,000; polymorphonuclear neutrophils, 83 per cent; lymphocytes, 13 per cent; monocytes, 3 per cent; coagulation time, ten minutes; bleeding time, fifty-five minutes; tourniquet test, positive; clot retraction, none.

7. *Carcinoma.*—Both cases showed a typical blood picture of thrombocytopenic purpura. Case 1 showed widespread metastases of the long bones and in the other case, no evidence of metastasis of the long bones could be found. Purpura was found in one patient who had a dermoid cyst of the ovary. After the removel of the tumor, the purpura cleared up. The relation of purpura to the ovarian function is discussed by Meyerstein.[41]

8. *Typhoid Fever.*—The blood picture in this case is that of the thrombocytopenic variety. The patient showed a very marked hemorrhagic tendency with the development of the purpura, and died from exsanguinating intestinal hemorrhages which numerous transfusions failed to stop.

C. CHRONIC THROMBASTHENIC PURPURA

Glanzmann[42] differentiates this condition from hemophilia by the presence of a prolonged bleeding time and absence of clot retraction. Van der Zande[43] observed a family in which thrombasthenia was present both in male and female members. The clot retraction was present but somewhat retarded. The two cases here reported occurred in a mother (Case 1) and a son (Case 2). They are mild bleeders and have had attacks of epistaxis, purpura, and ecchymoses. It is interesting that an abdominal operation in one case was

not followed by undue hemorrhage. The coagulation and bleeding times were distinctly prolonged in both. Case 1: hemoglobin, 95 per cent; red blood cells, 6,000,000; white blood cells, 8,000; platelets, 180,000; polymorphonuclear neutrophils, 50 per cent; lymphocytes, 42 per cent; monocytes, 6 per cent; coagulation time, twenty-six minutes; bleeding time, six minutes; tourniquet test, negative; clot retraction, normal. Case 2: hemoglobin, 90 per cent; red blood cells, 5,400,000; white blood cells, 13,000; platelets, 240,000; polymorphonuclear neutrophils, 59 per cent; lymphocytes, 36 per cent; monocytes, 3 per cent; coagulation time, sixteen minutes; bleeding time, ten minutes; tourniquet test, negative; clot retraction, normal.

D. HYPERTENSION AND NITROGEN RETENTION (TABLE XVI)

Anemia is always present in such cases as reported by Berg[44] and others. The occurrence of purpura is rather unusual in such cases although other forms of hemorrhages (brain, retina, nose, stomach, and intestines) are not unusual. The platelets are normal or even increased, and the clotting time is prolonged in most of the cases.

In the six cases with purpura which came under observation, five showed a diminution of the blood platelets (50,000-110,000), a moderately increased bleeding time, and normal clot retraction. The tourniquet test was positive in all. Case 6 showed very marked arteriosclerotic changes, which were partly responsible for the hemorrhagic condition (so-called arteriosclerotic purpura).

E. JAUNDICE (TABLE XVII)

The bleeding tendency in certain cases of jaundice is attributed to some fault in the coagulation of the blood as a result of some change in the calcium factor (Morowitz, Lee and Vincent). Purpura is not a common symptom and was noticed in only four cases. The platelets do not seem to play an important rôle in its production. In Case 4, there was a marked prolongation of the bleeding time and coagulation time. The tourniquet test was positive in all cases. The condition of the capillaries possibly is also affected by the jaundice and may be partly responsible for the prolonged operative bleeding time (Leschke) in addition to the altered coagulability of the blood.

TABLE XVI

HYPERTENSION AND NITROGEN RETENTION

NO.	NAME	HGB.	RBC.	WBC.	PL.	PN.	L.	M.	CT.	BT.	TT.	CR.
1	H.G.	70	4,2	10	50	70	27	4	10	5	+	Normal
2	L.D.	54	2,1	5	60	68	29	2	10	10	+	Slight
3	I.B.	55	2,9	5	105	64	31	4	8	2	+	Normal
4	I.M.	42	2,1	4	105	70	22	3	15	3	+	Normal
5	D.W.	54	2,5	16	110	91	1	1	10	15	+	Normal
6	J.R.	70	3,6	12	190	68	27	5	18	6	+	Normal

TABLE XVII

JAUNDICE

NO.	NAME	HGB.	RBC.	WBC.	PL.	PN.	L.	M.	CT.	BT.	TT.	CR.
1	B.K.	55	2,7	7	126	80	19	1	30	3	+	Normal
2	H.T.	90	4,7	8	175	83	12	4	5	1	+	Slight
3	S.S.	92	6,0	8	260	78	21	1	12	5	+	Normal
4	A.N.	65	3,8	12	310	80	14	6	24	12	+	Normal

TABLE XVIII

SCURVY

NO.	NAME	HGB.	RBC.	WBC.	PL.	PN.	L.	M.	CT.	BT.	TT.	CR.
1	E.T.	40	2,2	4	220	67	30	3	7	3	+	Normal
2	A.M.	75	3,9	7	290	74	22	4	12	1	+	Normal
3	E.W.	58	2,7	4	480	68	29	2	4	8	+	Normal

TABLE XIX

SCHOENLEIN-HENOCH'S PURPURA

NO.	NAME	HGB.	RBC.	WBC.	PL.	PN.	L.	M.	CT.	BT.	TT.	CR.
1	H.P.	100	5,2	11	190	53	36		7	2	+	Normal
2	P.G.	80	4,0	10	200	73	20	5	5	3	+	Normal
3	M.L.	94	4,8	6	210	56	42	2	9	2	+	Normal
4	S.S.	80	3,8	7	230	57	36	6	7	3	+	Normal
5	C.M.	98	4,8	5	240	66	22	5	12	3	+	Normal
6	J.B.	80	4,4	8	240	42	55	3	9	3	−	Normal
7	M.D.	88	4,5	8	410	64	27	4	12	1	−	Normal
8	F.R.	48	3,3	21	420	87	7	6	6	3	+	Normal
9	S.P.	90	4,6	17	610	87	10	2	12	2	−	Normal

F. SCURVY (TABLE XVIII)

Except for the positive tourniquet test, the blood picture, in cases of scurvy, is normal. Anemia may occur after excessive hemorrhages of long duration. The diagnosis of this condition depends upon the history of the deprivation of the individual from foods containing vitamine C.

G. SCHOENLEIN-HENOCH'S PURPURA OR PELIOSIS RHEUMATICA (TABLE XIX)

Schoenlein (1839) described a form of purpura associated with a joint involvement, and in 1868 Henoch described similar cases with colic and intestinal hemorrhages. The purpuric manifestations are sometimes associated with erythema and urticaria which rarely occur with thrombocytopenic purpura. The etiology of this condition is still unknown. Frank[45] believes that it is due to some process of autointoxication, probably from split protein products resembling histamine, producing a toxic effect on the capillaries. This disease has also been attributed to tuberculosis. Castex,[46] in the study of two cases, noticed the symmetrical distribution of the purpura and found definite changes in the sympathetic nuclei.

The blood picture is normal except for the positive tourniquet test. The blood platelets are not altered either quantitatively or qualitatively, and only offer confusion with the true thrombocytopenic purpuras when the blood examination is omitted.

H. PURPURA FULMINANS

Very little is known about the cases of purpura fulminans, which usually occur in children. The blood picture reported in our case was in an adult. The condition may be a fatal form of the Schoenlein-Henoch's purpura group. Hemoglobin, 84 per cent; red blood cells, 5,200,000; white blood cells, 19,000; platelets, 250,000; polymorphonuclear neutrophils, 86 per cent; lymphocytes, 10 per cent; monocytes, 4 per cent; coagulation time, seven minutes; bleeding time, two minutes; tourniquet test, positive; clot retraction, normal.

SUMMARY

An analysis of the blood picture of the cases studied shows that purpura may be divided into three main groups: (1) Purpura characterized by a diminution of the blood platelets with or without alteration of their functions; (2) purpura due to some change in the function of the platelets without a diminution of their number; (3) purpura due to changes in capillaries.

1. The blood picture in the first is diagnostic in cases of acute and chronic thrombocytopenic purpura. These are characterized by diminution of the blood platelets with or without alteration of their function. Anemia is present after hemorrhages only. The number of blood platelets does not seem to bear any definite relation to the bleeding time, but occasionally this property may not be altered. Coagulation time is normal or slightly prolonged. The tourniquet test is exceptionally absent. The clot retraction is always impaired except in rare instances. The diagnosis of this condition can be definitely made from the blood picture when no cause is found to account for the hemorrhagic tendency. This condition is an acute or chronic constitutional disturbance, as a result of either altered blood platelet formation, or increased blood platelet destruction.

The blood platelets are also diminished in acute and chronic aplastic anemias; anemia and leucopenia are also present. The anemia is far greater than can be accounted for by the hemorrhages. The diminution of blood platelets is always associated with qualitative changes.

The diminution of blood platelets may be associated with other diseases (symptomatic thrombocytopenic purpura). The blood picture is diagnostic in the leucemias, frequently in subacute bacterial endocarditis, when macrophages are present, and in pernicious anemia. Attention again must be called to the fact that some cases of subacute leucopenic myeloblastic leucemia may be confused with thrombocytopenic purpura unless a careful differential blood examination is done for the identification of the premature myeloid cells, such as myeloblasts and myelogones. The splenomegalic conditions associated with purpura require other examinations for their identification. Cases of tuberculosis, carcinoma, subacute endocarditis, drug poisonings, and typhoid fever closely resemble thrombocytopenic purpura, but require special clinical observations for their identification. As a rule the purpura is not the outstanding feature in such cases.

2. The second group, chronic hereditary thrombasthenic purpura, is rare, and still requires further study before it can be definitely said that the blood platelets are wholly responsible for the condition. All the cases so far reported seem to be inconstant in the blood findings with the exception of the normal platelet count.

3. The third main group is purpura due to conditions which affect the capillaries. Schultz[47] classifies all the conditions as athrombopenic purpura, but it is far outnumbered by the thrombocytopenic purpuras. The various conditions which we have found that may affect capillaries are jaundice, hypertension associated with nitrogen retention, vitamine deficiencies, and Schoenlein-Henoch's purpura, the cause of which is not known. Most of them

are characterized by no change in the number of blood platelets, or by qualitative changes.

In hypertension and nitrogen retention the blood platelets may also be diminished. Subacute bacterial endocarditis also shows evidence of marked capillary disturbances as shown by the frequent appearance of petechiae and a positive capillary resistance test. Case 4 (Table XVI) showed no blood diminution of the blood platelets, but presented marked purpuric manifestations.

CONCLUSIONS

1. The ordinary blood examination is insufficient for the differentiation of the various types of purpura. In addition the blood platelets must be counted and their functions studied in each individual case.

2. The blood picture shows three main groups of purpura:

(a) Thrombocytopenic purpura. Purpura is either the predominating symptom as a result of a diminution of the blood platelets, or it may be symptomatic.

(b) Chronic thrombasthenic purpura (rare). The blood platelets are normal in number but show qualitative changes.

(c) Purpura as a result of alteration of the capillaries due to numerous causes. In a few cases there may be an additional disturbance of the blood platelets, as in uremia and subacute endocarditis.

3. A classification is presented as a result of a systematic study of 172 cases showing purpura as a primary or secondary symptom.

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DISCUSSION

Dr. Reuben Ottenberg.—With regard to the tourniquet test it seems to me that blood pressure has something to do with it. If a standard time and pressure are used, the method is perfectly satisfactory as petechial spots do not occur in normal individuals. If the pressure is excessive petechial spots are obtained in all individuals. Leave the tourniquet on for three minutes at a pressure intermediate between the systolic and the diastolic blood pressure. Under those conditions normal persons do not show petechial spots, and abnormal ones do.

Dr. Reed Rockwood.—What method did you use for the determination of blood platelets and what is its approximate error? Have you made any observations upon the morphology of the platelets in the thromboeytoplenic form of purpura by means of the ultramicroscope? A very few observations which I made in this group of cases show that there is a distortion of the structure of the platelets which is made visible by this method of study.

Dr. Rosenthal (closing).—The time allowed did not permit me to take up the counting of the blood platelets and their morphology. As to their enumeration we have used two methods. The first is the use of the 3 per cent sodium citrate similar to that of counting

red blood cells, and the second method is the counting of blood platelets from citrated plasma. The morphology of the blood platelets are not important as a differential factor. In purpura hemorrhagica, leucemia, pernicious anemia, and in splenomegalic conditions, the platelets were large in size and showed the same characteristics in the citrated plasma as in the stained smears where the blood platelets could be found. In regard to the tourniquet test, Lewis recommends the constriction of the arm with the sphygmomanometer at 70 mm. of mercury for three minutes. This method reveals more positive reactions than the ordinary rubber tourniquet.

ANEMIA AS A FACTOR IN THE SEDIMENTATION TIME OF ERYTHROCYTES*

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THE literature upon the sedimentation time of erythrocytes is extensive and is growing rapidly. This literature divides itself naturally into two groups of articles, one dealing with the clinical, the other with various theoretical aspects of the problem. The correspondence between these lines of study is often very far from satisfactory.

There are many factors which must affect the rate at which red blood cells settle in any given instance. These include, automatically, the viscosity of the plasma and the relative densities of the cells and plasma. Changes in these do not account satisfactorily for many of the observed facts, and emphasis has been laid upon variations in the proteins of the blood and electrical relationships between the cells and the fluid in which they are immersed to explain many of the abnormalities observed in clinical conditions.[1, 2]

The group of cases where clinical interest has centered most keenly is that where there is absorption of abnormal products from bacteria or from cell disintegration into the system. Studies have included most of the infections, extensive breakdown of tumor tissue, pregnancy, etc. In almost all of these it has been shown that blood cells settle more rapidly than they usually do.[3]

A second factor which alters the rate, and which, in the opinion of the authors, has not been sufficiently emphasized in current American clinical literature, is the concentration of the red cells. It has been repeatedly shown, particularly by German investigators, and has been satisfactorily discussed in articles by Rubin and various collaborators,[4, 5, 6, 7] that there is a slow sedimentation time in polycythemia and a rapid one in anemia. This fact is certainly not properly allowed for in many of the presentations of clinical material which have appeared. In many of the diseases discussed, anemia is not infrequently present, and if the effect upon the infectious process is to be studied adequately some attempt must be made to differentiate the effect of this secondary factor from that of the primary one. Even when mention

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From the laboratories of the Clifton Springs Sanitarium and Clinic.

is made of anemia as a possible cause for some variations from the normal, it is only an extreme degree of anemia which is considered; that moderate changes in the blood count may have an appreciable effect upon the sedimentation rate has been more or less completely ignored.

There is apparently very little experimental work which bears upon this problem. In 1925 a single experiment upon the effect of diluting red blood cells with their own plasma was described in the Clifton Medical Bulletin.[2] In 1926 Branch, Pien, and Tung found that if washed corpuscles were suspended in normal saline solution the sedimentation time was greater when the volume of corpuscles was relatively greater.[5] In the present year Rubin and Smith have reported experiments very similar to those described below, demonstrating that artificially produced variations in the cell volume cause corresponding variations in the sedimentation rate.[7] As a consequence of their work, they conclude that more attention should be paid to slight variations in cell volume than is usually given to them.

Our own experiments furnish data upon the effect of relatively small differences in cell volume upon the sedimentation time. They were carried out by the method used by Friedenwald, which is slightly modified from that of Linzenmeier.[9] Blood was drawn from the arm vein into a record syringe containing a 5 per cent solution of sodium citrate; enough blood was taken so that in the final mixture there were four parts of blood for each part of solution. For the control reading 1 c.c. of blood was transferred to a glass tube, and the time it took the top of the column of red blood cells to settle 18 mm. was noted and recorded as the sedimentation time. In some experiments a measured amount of the blood and citrate mixture was poured into small stoppered graduated cylinders, and, as soon as the cells had settled sufficiently, a definite amount of plasma was pipetted off. The remainder was then thoroughly mixed, 1 c.c. placed in the standard tube, and the sedimentation time determined as described above. In several cases tubes were allowed to stand as controls and were shaken up without removing any of the plasma; these showed the same sedimentation rate as did the blood measured immediately after it was drawn. Besides these experiments where the relative cell volume was increased, there were others in which it was diminished. In these a measured amount of additional plasma, obtained by centrifuging a portion of the blood, was added to a measured amount of blood. The specimens were then thoroughly mixed, and the sedimentation time determined. As controls upon this technic, one blood sample was treated as follows: 5 c.c. of blood was allowed to stand in a graduated shaking cylinder until it was possible to remove 1 c.c. of plasma. This was replaced by 1 c.c. of plasma from which the cells had been separated by centrifugalization. The sedimentation time was 193 minutes, while that determined on the whole blood immediately after it was obtained was 191 minutes.

The results of our experiments upon normal laboratory workers and patients suffering from different diseases are given in Table I. It will be seen that there was a perfectly definite change in the sedimentation time when the ratio between plasma and cells was varied only slightly, for a change of 0.5 c.c. in a total volume of 5 c.c. amounts only to about 10 per cent. In Table

II are given the results of blood counts done at approximately the same time as that at which the sedimentation test was carried out, and the clinical diagnoses on the abnormal subjects studied. If the figures for the red cell counts are compared with the results in Table I, it is plain that in some instances rates which would be at least classed as suspicious were obtained upon subjects with normal sedimentation rates when the number of red cells was reduced to figures which would not indicate a very severe grade of anemia. Case 5, when an artificially produced anemia of about 3,700,000 cells per c.mm. was present, showed a sedimentation time of sixty-one minutes, which is usually accepted as distinctly abnormal by this method, and Case 7, which had a sedimentation time of 261 minutes when the red blood count was 5,450,000 per c.mm., gave a value of only ninety-one minutes in the specimen which corresponded with a count of approximately 3,940,000 per c.mm. A similar marked change can be demonstrated when the experiments made the blood more concentrated. For example, Case 10 showed a settling time of seventy-eight minutes, a value which has not infrequently been considered

TABLE I

SEDIMENTATION TIME (IN MINUTES)

NUMBER		5 C.C. BLOOD PLUS PLASMA							5 C.C. BLOOD MINUS PLASMA			
CASE	SERIES	0.0 C.C.	0.5 C.C.	1.0 C.C.	1.5 C.C.	2.0 C.C.	2.5 C.C.	5.0 C.C.	0.5 C.C.	1.0 C.C.	1.5 C.C.	2.0 C.C.
1	14	442	281	225	174	144	—	—	—	—	—	—
2	13	274	160	140	112	101	—	—	—	—	—	—
3	11	233	—	193	—	—	85	61	—	—	—	—
4	10	496	358	230	148	115	—	—	—	—	—	—
5	12	117	100	78	—	61	—	—	—	—	—	—
6	16	232	182	143	124	111	—	—	—	—	—	—
7	5	261	—	152	—	91	—	—	—	—	—	—
8	6	58	—	37	—	27	—	—	—	133	—	—
9	3	95	—	—	—	—	—	—	—	530	1961	—
10	2	78	—	—	—	—	—	—	102	120	—	415
11	8	43	—	—	—	—	—	—	54	59	69	76
12	4	45	—	—	—	—	—	—	55	76	—	520
13	7	7	—	—	—	—	—	—	12	17	—	21

TABLE II

CASE	SERIES	SEX	BLOOD COUNT				DIAGNOSIS
			ERYTHROCYTES NO.	LEUCOCYTES NO.	HEMOGLOBIN %	NEUTROPHILES %	
1	14	M	—	—	—	—	Normal
2	13	M	5,500,000	6,400	87	50.2	Normal
3	11	M	5,350,000	7,400	85	48.0	Normal
4	10	M	5,180,000	10,200	97	75.0	Normal
5	12	M	5,030,000	8,000	93	45.1	Normal
6	16	F	4,400,000	8,800	79	65.0	Normal
7	5	M	5,460,000	23,600	106	22.2	Diabetes; furunculosis
8	6	M	3,950,000	11,800	80	70.4	Sprue
9	3	F	3,930,000	7,000	71	49.0	Acute trigonitis
10	2	M	3,700,000	9,800	68	67.3	Pernicious anemia
11	8	M	2,850,000	25,600	29	91.0	Carcinoma of stomach; secondary anemia; Glycosuria
12	4	F	4,050,000	8,000	71	62.7	Sarcoma, metastic, axillary glands and bladder
13	7	M	3,250,000	15,600	47	75.7	Abscess of lung

abnormal when the blood count was 3,700,000 per c.mm. When the specimen was concentrated to approximately 5,180,000 cells, the time was 120 minutes, or approximately normal.

It does not seem best to place too great emphasis upon such calculations for several reasons. In the first place it cannot be said with certainty that such dilutions and concentrations correspond to the changes that may take place in the body in anemia, although it seems to us that this plan serves as a method for studying the effect of such changes by themselves better than any other experimental technic which can be devised. In the second place such calculations place all of the emphasis upon changes in the number of cells, while it is not impossible that the variations in sedimentation time correspond with changes in cell volume rather than cell concentration. The number of cells is the most important factor which determines cell volume, but it is not the only one upon which it depends; the average size of the cell is also a factor. A third reason for not laying too much stress on the results is that changes in cell volume or of cell concentration may apply only to the methods used for measuring the sedimentation time. It is almost tacitly assumed, it seems to us, that the rate at which the column of red cells settles out from solution is to some extent a measure of the rate at which the average red cell would settle. From our experiments it seems almost certain that this is not entirely true. A fact that may apply only to a method of measuring a phenomenon may have little or no physiologic significance. On the other hand changes in cell volume, or cell concentration, almost certainly does affect any of the methods of estimating the sedimentation time now in use, as has already been shown by Rubin for one of the other standard technics, and therefore must be taken into account when such readings are interpreted.

There are a number of possible plans for correcting the "error" produced by variations in cell concentration. One is that discussed by Rubin and Smith[7] who have suggested that results be expressed as the ratio between the amount of sedimentation in one and twenty-four hours. This is approximately, although not exactly, an expression of the ratio between sedimentation rate and cell volume. Another method would be to determine an average "normal" sedimentation rate to correspond with different cell concentrations, hemoglobin contents, and cell volumes. This is obviously impossible because the differences among normal subjects with approximately the same blood picture is so marked, a point which has been repeatedly brought out in the literature and which is clearly shown by the tables. Another thing which a study of Table I reveals is that there is no constant quantitative relationship between the percentage of changes produced in cell concentration (or cell volume) and the sedimentation rate. It would not be possible to determine the sedimentation time and blood count, or cell volume, on a given patient, and calculate what the rate would be for a different blood count. A number of other plans for working out the effect of cell concentration in a given case were tried, but none of them can be described as wholly satisfactory. Our technic which was carried through several times was as follows: the cell volume was determined on the blood as drawn and on each specimen which had been diluted with plasma by centrifuging at 2500 revolutions per minute

for thirty minutes; cells from each of the diluted specimens were then mixed with plasma in the same proportions in which they were found in the initial blood, and sedimentation times determined. On the average the results agreed very well with those obtained on the initial blood specimen, but in several instances such marked discrepancies were found that the method cannot be recommended. The sources of error include mistakes in technic (the high viscosity of the cells makes accurate measurement very difficult), a possible effect of standing, which was probably slight, and electrical changes induced by the excessive manipulation. In our opinion this last is the most serious source of error. A better plan would be to allow a measured amount of blood to stand until the cells have partially settled and then remove a portion of the plasma calculated to produce the desired cell concentration. Such a technic would avoid the more important sources of error found when cells and plasma were separated, and would be liable only to that produced by standing—an error which our own results and those of others seem to show is relatively small. It must be borne in mind, of course, that such a correction may not bear any relationship to conditions in the body.

There is a distinct relationship between the rate of sedimentation of erythrocytes on the one hand and their concentration in the blood on the other. Rather small variations from normal blood counts may apparently produce quite marked differences in the sedimentation rate as usually measured. When the sedimentation time is used clinically, this fact should be kept in mind, for other factors cause marked deviations from the normal, and it is in these other factors that the clinician is primarily interested in his application of the test.

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STUDIES OF SEDIMENTATION OF ERYTHROCYTES*

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TECHNIC

SOME of the most ancient writers in medical literature refer to the difference in the sedimentation time of blood cells in specimens of drawn blood. Galen noticed, particularly in acute inflammatory processes, that the cells became separated from the plasma even before coagulation had set in. Later John Hunter, Virchow, and others made the same observations. The clear yellowish serum that was left by the sinking away of the red cells was called the "crusta inflammatoria" or "crusta phlogistica," and was regarded as an exceedingly important sign. The color, thickness, and consistency of the crusta inflammatoria were considered significant in the diagnosis, treatment, and prognosis of the disease. As the practice of bleeding patients fell into disrepute, the sedimentation of the blood cells and the crusta inflammatoria were forgotten.

Biernacki, in 1897, reported a series of seventy-five sedimentation tests. He concluded that the test might be of some value in the diagnosis of diseases. Robin Fahraeus, in 1917, rediscovered this phenomenon, and his observations were published in articles in *Hygeia* in 1918, and in the *Biochemische Zeitschrift* of the same year. He believed the condition might be used as a test for pregnancy as there was a marked difference between the sedimentation of the blood cells in parturient and nonparturient women. He also called attention to the fact that in cases of infectious diseases and malignant tumors as well as during pregnancy there is acceleration of the sedimentation rate.

Fahraeus continued to work on this problem and published an article in which he advanced the theory that the increased sinking velocity of the red cells (or increased sedimentation) which has been shown to exist during pregnancy, and many diseases is dependent on either an increased agglutination of the corpuscles or on a decrease in their number. The agglutination here in question is identical with the rouleau formation of the red blood cells, and the increased agglutination is only an exaggeration of the normally existing phenomena. Fahraeus also noted that in the cases in which the agglutination was increased, there was a corresponding increase in either the serum globulin or the fibrinogen or both.

The etiologic factor in blood sedimentation has been extensively investigated. An increase or change in the electrical charge of the red cells has been held responsible by certain investigators. An increased cholesterol

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content, changes in viscosity of the blood plasma, variations in the erythrocyte count, and certain chemical changes in the blood have been described as causative factors, but none has been definitely proved.

METHODS OF ERYTHROCYTE SEDIMENTATION

Many of the methods of carrying out blood sedimentation tests are essentially the same, but they differ in the amount of material used, end point, size of tube, time of reading, and collection of the specimens.

Waugh using Linzenmeier's method collects 0.8 c.c. of blood in a special graduated syringe containing 0.2 c.c. of a 5 per cent sodium citrate solution. He then transfers this to a small sedimentation tube about 6.5 cm. high and 0.5 mm. clear diameter, with calibration marks at 1.0 c.c., and at distances of 6, 12, 18, and 24 mm., respectively, from the 1.0 c.c. mark. The sedimentation tube is filled exactly to the 1 c.c. mark with the citrated blood, and the line of separation between the blood corpuscles and the plasma is noted as it reaches the various marks; the time that this takes is reported as the sedimentation time.

The Westergren and Fahraeus technic, as described by Friedlaender, differs considerably from the Linzenmeier method. With a syringe holding 0.4 c.c. of a 2.5 per cent sodium citrate solution, blood is drawn from a vein to the 2 c.c. mark. The blood and citrate are well mixed, and then transferred to a 1 c.c. pipette up to the zero mark. After one hour the column of erythrocytes is marked at its line of separation from the plasma and is measured in millimeters. A plasma column of from 3 to 15 mm. in one hour is considered normal. Westergren, in a recent article, describes a more improved technic, although the fundamental principles are the same.

Zeckwer and Goodell collect 8 c.c. of blood in a graduated centrifuge tube containing 2 c.c. of 3 per cent sodium citrate solution. The mixture is allowed to stand, and the readings of the heights of the red cells are made at the end of one hour.

Cooper has combined the methods of Fahraeus, Zeckwer, and Goodell. He draws the blood into a bottle into which 3 drops of 20 per cent oxalate has been allowed to dry. This anticoagulant is sufficient for from 15 to 20 c.c. of blood and is usually used in conjunction with the taking of a specimen of blood for chemical analysis. Into a dry 15 c.c. graduated test tube is placed 5 c.c. of blood. This is allowed to stand in a rack, and readings are made at the following periods: five, ten, fifteen, thirty, forty-five, and sixty minutes, to note the level to which the cells have settled. The blood is centrifugalized at high speed for ten minutes, and the reading which is taken corresponds to that of the cell volume. This reading is practically identical with that obtained by twenty-four hours' sedimentation.

The technic of the test as used by Rubin is as follows: Into a sterile 2 c.c. record syringe a solution of 3.8 per cent sodium citrate solution is drawn to the 0.4 c.c. mark. Blood is then aspirated from a vein in the arm to the 2 c.c. mark, giving a dilution of 1:4. After this has been thoroughly mixed, it is drawn into long serologic pipettes graduated to hundredths, placed on a rack, and the layer of clear plasma observed at the end of one, two, and twenty-

four hours, the readings noted directly in percentage of 1 c.c. He considers the two-hour reading as the most significant one.

After the literature had been carefully reviewed for different methods and many of them tried, I decided to modify the technic described by Westergren and Rubin. Since it was evident from preliminary work that it was unnecessary and inconvenient to use special syringes containing citrate or oxalate solutions and since it is difficult to obtain many tubes of uniform diameter, I used the ordinary 1-c.c. serologic pipettes. The problem was to obtain a great many specimens in a short time, hence, blood was aspirated from the median basilic vein, using a dry 5 c.c. syringe. Three and five-

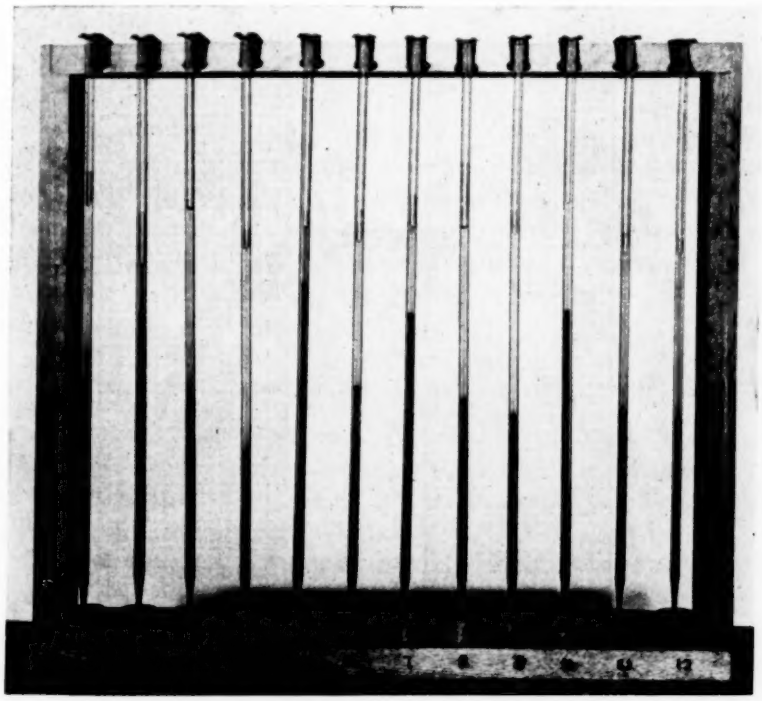

Fig. 1.—Set-up for sedimentation test.

tenths cubic centimeters of the blood was transferred to a graduated centrifuge tube containing 0.5 c.c. of 1.6 per cent sodium citrate solution. The tube was then inverted several times so as to insure a thorough mixing of the blood with the solution. Exactly 1 c.c. of the mixture was then drawn up into a 1-c.c. serologic pipette graduated to hundredths, placed in the special rack designed by Westergren, and the separating line between the plasma and cells observed at the end of one, two, and twenty-four hours (Fig. 1). The reading obtained by using the graduated pipettes represents a definite percentage of the total volume of blood used.

There has been a great deal of confusion in the method of reporting the results of the sedimentation reaction. Some report the time that it takes the top of the column of red cells to reach a certain level, others the dis-

tance that the cells fall in a certain time. After several hundred sedimentation tests had been charted (when the reading had been recorded every hour for several hours), it was observed that the most constant drop in the curve occurred between the first and second hour. Rubin, Morriss, and others observed this fact and considered it the most significant part of the curve. Tubes of different diameters when used with the same blood give the same

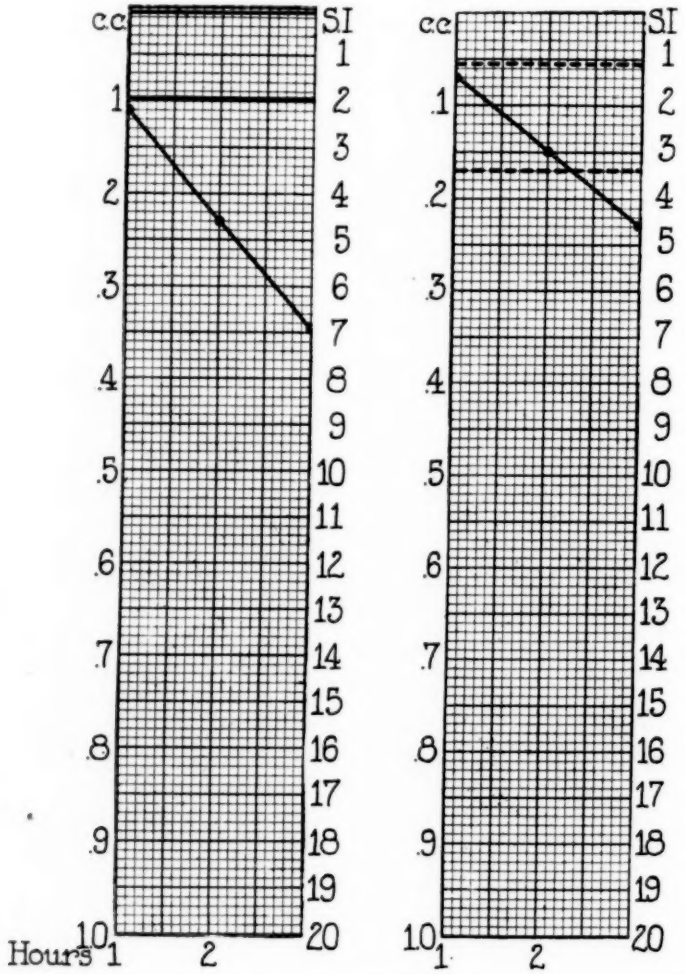

Fig. 2.—Determination of sedimentation index. Values between heavy lines indicate range of normal males. Values between dotted lines indicate range of normal females. *S.I.*, sedimentation index.

results. With this fact in mind, I devised a method, based on the work of Gilbert, of reporting the sedimentation reaction which would be a combination of time and distance. This was called the sedimentation index. This seems to me to be a more nearly correct usage of the term index than that of Cutler who in using the term sedimentation index refers to the total sedimentation of the red blood cells at the end of sixty minutes, expressed in

millimeters. According to my method, the sedimentation index can be obtained by the use of either a graph or a table.

The graph paper is standard, authorized by the American Medical Association for its journals and is ruled in one-half inch squares each of which is subdivided into twenty-five equal squares. However, any paper ruled in equal squares would serve as well. The ordinates represent time, each large square indicating a half hour. The abscissa represent volumes of plasma appearing above the falling column of cells, hence, the distance the erythrocytes fall. Each large square represents a fall of 0.05 c.c.

The sedimentation index (Fig. 2) is obtained by projecting the line drawn between the plotted point of the first and second hours, onto a scale drawn as a perpendicular line two squares to the right of the second-hour point or so that the horizontal distance from the first-hour point to the second-hour point is equal to the distance from the second-hour point to the scale. The scale is enumerated with 0 on the horizontal line marked c.c., and the integral numbers follow in order downward, each large square representing one unit. The reading is recorded in units and decimals thereof. Thus, a rapid sedimentation rate gives a high sedimentation index.

It will be noted that the method presented here differs from Gilbert's chiefly in that he determines the sedimentation index by drawing a straight line from the 0 point paralleling the greatest drop in the curve during the first hour, to a scale on the first-hour line.

SUMMARY

A critical study of methods employed in the sedimentation test has been made. By combining and modifying several tests, a simple and expedient test has been devised. A simple method of reporting results has been proposed, the results being given in terms of a sedimentation index. Average normal values for adult males are from 0.1 to 2.0 and for adult females from 1.1 to 3.4, although it must be stated that in occasional instances, there are unexplained variations from the average normal values given. Sedimentation tests by this method can readily be compared with those done and reported by other methods described in the literature.

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DISCUSSION OF PAPERS BY HUBBARD AND GEIGER AND HUNT

Dr. Carl Spohr.—We have been doing some extensive experimenting in our laboratory on this subject with the view of preparing a paper for this meeting. We have discovered that there are a great many factors which require detailed study. Our method has to do with Sahli tubes. We have used undiluted blood to which an anticoagulant has been added. We place oxalated blood up to the 100 mark in the Sahli tube. It has been our experience that an increased amount of oxalate retards sedimentation. A number of determinations have been made with other anticoagulants. We can state that excessive amounts of anticoagulants will markedly retard sedimentation. Sufficient blood is drawn for several determinations and one portion is allowed to stand from twelve to twenty-four hours in the ice chest. It gives a decidedly lower reading. Readings are from 5 to 10 per cent lower than the readings obtained by making the readings immediately after the blood has been withdrawn. If we add citrate salt solution, the cell volume is markedly higher. We have studied a number of cases in gynecology and have obtained some valuable results. Our method was to use a Sahli tube, 2 mg. to the c.c. up to the 100 mark; readings are made in five, ten, fifteen, thirty, forty-five, and sixty minutes. These are graphed; the cell volume is also determined.

We have found that the sedimentation velocity in acute active cases is markedly increased, and that we can get satisfactory readings by this method in about thirty minutes.

We have just begun to study this problem and we expect to continue this until we get more definite information. I have given you some of the points which we have really noted that may be of value to you in the future.

Dr. Asher Yaguda.—About two or three years ago when sedimentation was fast becoming popular in this country I started to do some work with it. I learned that many factors varied the sedimentation time. I took a number of normal individuals who seemed to be in good health and found one of the factors to be exercise. Exercise cut the sedimentation time in half, and a heavy protein diet did the same thing. Some of the other factors were the type of coagulant, and the temperature of the room in which the sedimentation time was taken.

Dr. Herman Sharlit.—I simply arose to ask, in view of the fact that one of the simple factors would be the specific gravity, if that was specially investigated and if so what the inference was.

Dr. H. R. Brown.—I would like to ask a question about the size of the red cells in these experiments. Just what is the practical application in cases of infection? I should like to hear an expression on that subject.

Dr. William G. Exton.—I would like to ask also if there is any available data on the various elements that must enter into this time drop, such as the specific gravity of the particular cells and particular plasma.

Dr. Roger S. Hubbard.—It seems to me that the principal points which should be borne in mind when studies of the sedimentation rate are applied clinically have been very well brought out in the discussion. There are a number of different methods which may be used, some of which are better than others, but which all measure the same things and are more or less influenced by the same factors. The specific gravity of the blood, the amount of exercise which the patient has taken prior to the test, and the degree of anemia present are among what may be called "secondary factors" which certainly influence the sedimentation rate but are not the ones in which the clinician is interested. A few experiments were carried out to determine whether the effect of anemia could not be overcome. Specimens of blood were centrifugalized, and the relationship of the volume of cells to the total volume determined. Artificial anemia was then produced as described in the paper, the anemic blood centrifugalized, and the cells and plasma mixed in the proportions in which they were present in the original samples. Sedimentation times were then determined on the mixture of cells and plasma. The average of the results obtained agreed well with the sedimentation times determined on the blood as drawn. In a number of individual studies, however, the differences were marked, and a method for ruling out the effect of low cellular concentration based upon these experiments, therefore, cannot be recommended.

Dr. A. H. Sanford.—In all of the discussion it seems to me that we have raised the question whether we are not simply dealing with some biologic phenomena that can have, after all, very little real diagnostic use. I do not want to offer this as a criticism of Dr. Hunt's work, because he is still working on his problem. All phases of it must be studied. At present I think we are being rather hurried into reporting the sedimentation rate of erythrocytes as a diagnostic procedure. The test seems to be principally looked forward to by the gynecologists. That is merely my impression. The gynecologists are insisting that it is a very important test, and marked changes do occur in those conditions that have been mentioned. However, there is a considerable difference in the sedimentation rate of women's blood as compared with that of men. The physiology of the process needs more study, I am sure.

Dr. G. B. Kramer.—It seems to me that the present status of the sedimentation test of erythrocytes is not so much for diagnosis as it is of some aid for the surgeon in deciding when to operate infectious cases. In our hospital some surgeons believe that in pelvic infections where the sedimentation rate is accelerated, operation is contraindicated. It seems to have more value as an aid to prognosis than to diagnosis.

THE MICROSCOPIC SLIDE PRECIPITATION TEST FOR SYPHILIS*†

By Francis B. Johnson, M.D., Charleston, S. C.

SINCE the introduction of the Kahn precipitation test, many investigations have been made of its usefulness as compared with the Wassermann in the serologic diagnosis of syphilis.

We have now ample evidence of its great value as a diagnostic procedure and of its greater saving in time, material, and expense.

One of the chief difficulties with the Kahn precipitation test is in the reading of the doubtful and slightly positive reactions. The ability to record correctly the results, with the unaided eyes, depends upon a certain number of factors, of which normal visual perception is of the utmost importance; when there is any defect in vision the poor results are evident. Correction by properly fitted glasses will, of course, obviate this, but even so, considerable experience and practice are required. The holding of the test tube at a certain angle, the degree of light and shadow that strike the tube, is of such importance that each individual has a special light and position that he prefers to make his reading with.

It hardly seems necessary to mention the difficulties of the Wassermann, for we are more familiar with them and with the fact that it depends upon a greater number of variable factors and technical details. The Wassermann offers, however, little difficulty in the reading of end-results when made by the method of Kolmer. In regard to the care of technical details, it is essential to be as careful, and to have as thorough an understanding of what you are doing in one serologic test as in another. We should strongly condemn the idea that with the introduction of the Kahn test we have a method so simple and easy, that it can be done by any doctor in his office or by inexperienced individuals without the proper safeguards and controls.

The adaptation of the Kahn precipitation test to a method so as to be read by the aid of the microscope has been introduced by Kline and Young.[1] Lately they have added certain refinements in technical details, and it is claimed by them, that it is as specific as the Kahn and Wassermann tests, has the advantage over these methods, in that it is much simpler, requires less apparatus and serum, and is much easier to read than the Kahn test.

The technical details, as here given, are taken from Kline and Young[2] for the immediate slide precipitation test for syphilis.

TECHNICAL DETAILS

Microscopic slides, two by three inches, are thoroughly cleaned by rubbing with bon ami paste, which is allowed to dry, and rubbed off with a soft cloth. A wire loop, having a diameter of 12.5 mm. to 13 mm., wrapped

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with thread and bent at right angles to the stem, is used for making twelve paraffin rings on the slide, by dipping the loop in smoking paraffin, and touching the slide with the loop. All apparatus should be kept warm, about 70° to 80° F., and the air of the room should be fairly moist.

Serums are heated for a half-hour at 56° C. With a 1 c.c. pipette, graduated in $\frac{1}{100}$, 0.05 c.c., to 0.06 c.c., of each sera is placed in the center of a paraffin ring, allowing the tip of the pipette to touch the glass. Positive control, negative control, and salt control should be used.

Antigen should be prepared, titrated, and mixed for the proper dilution, according to the method of Kahn.[3] Antigen dilutions should be prepared just before pipetting sera, and are usually most satisfactory when used between ten minutes and a half hour after making up. In recent work it has been shown, by Kline and Young,[2] that a more satisfactory clear antigen is obtained after placing the cholesterolized antigen on ice for one hour, then filtering. Through the kindness of Doctor Kline, I have been able to check some of his recent antigen with mine, prepared in the same way, and have found it to be preferred to those that were filtered without chilling. One drop of antigen dilution is added to each sera by the use of a capillary pipette, dropping 0.0075 to 0.0085 c.c., to the drop. In earlier tests 0.015 c.c. was added. Each sera and antigen is mixed by the aid of the broad end of a wooden toothpick. After the completion of adding the antigen to the twelve rings, the slide is taken between the thumbs and index fingers of both hands and by a rocking circulatory movement further mixing is done for two to three minutes. Any spilling from the chamber makes the reaction unsatisfactory and that particular serum should be retested. Tests are better done in duplicate, by using different antigens, so as to have a check on the results.

All readings should be made by the use of the microscope with low power objective and 10 X eyepiece, with a dim light. Results are recorded in pluses, according to degree of clumping and size of clumps. A clear fluid is negative. Fine granular clumping is +; a fine flocculation is ++; a marked flocculation is +++; and a coarse flocculation is ++++.

COMPARATIVE RESULTS

In a series of 1500 tests, I have made a comparative study of my results of the microscopic slide precipitation test, by the method of Kline and Young,[1] with the three tube Kahn test,[3] and with the Wassermann test.

The Wassermann tests were made by the overnight fixation method of Kolmer,[4] using 0.1 c.c. and 0.05 c.c. of serum and 0.1 c.c. of serum control. With the Wassermann, inactivation was for fifteen minutes, and natural antisheep hemolysins were not removed. For the tube Kahn and the microscopic slide test, the sera was heated for one-half hour. The same antigens were used for the Kahn and slide tests and the dilutions were titrated and prepared according to the method of Kahn, but were made up separately. Readings were carefully checked by two, and sometimes three, experienced individuals.

In Table I are seen the results of fifteen hundred comparative microscopic Kahn tests, Kahn tube tests, and Wassermann (Kolmer) tests.

TABLE I

FIFTEEN HUNDRED COMPARATIVE MICROSCOPIC KAHN, TUBE KAHN, AND WASSERMANN (KOLMER) TESTS SHOWING AGREEMENTS

	TESTS	PER CENT
Agreement of all three	1335	89.0
Agreement of microscopic tests and Kahn	1445	96.3
Agreement of microscopic tests and Wassermann	1370	91.3
Agreement of Kahn and Wassermann	1442	96.1
Microscopic tests positive	536	35.7
Kahn positive	513	34.2
Wassermann positive	459	30.6

It will be noted that a uniform agreement of all three was found in 89 per cent. I can find no other comparison of the three methods at the same time, but it is evident from this that there is a fairly close agreement between the three. The agreement of the 96.3 per cent of the microscopic tests with the tube Kahn compares favorably with the findings of Kline and Young, in their preliminary report of 268 comparative microscopic tests with the Kahn tests in which they found an agreement of 97.7 per cent. The agreement of the microscopic tests with the Wassermann did not compare so favorably with that of Kline and Young, who in their first series found an agreement of 92.3 per cent, and in their later series, with improved antigen, an agreement of 97.6 per cent. This will be considered more fully in discussing the disagreements.

It is of interest to mention that my agreements of the tube Kahn with the Wassermann, in this series, was 96.1 per cent, whereas in a previously reported comparison of six thousand tests,[5] the agreement was 90.7 per cent. From the comparison of the positives by the three methods, it will be noted that the greatest percentage of positives is obtained by the microscopic test, and the least number of positives by the Wassermann.

In Table II are shown the disagreements of the three tests, including anticomplementary Wassermann tests, together with a comparison of those disagreements in which syphilis could be clinically diagnosed.

TABLE II

FIFTEEN HUNDRED COMPARATIVE MICROSCOPIC KAHN, TUBE KAHN, AND WASSERMANN (KOLMER) TESTS SHOWING DISAGREEMENTS

	TESTS	CLINICALLY SYPHILIS	PER CENT CLINICALLY SYPHILIS
Total Disagreements	165	127	76.9
Micro. Pos., Kahn Pos., Wass. Neg.	64	47	28.4
Micro. Pos., Kahn Neg., Wass. Pos.	8	8	4.8
Micro. Pos., Kahn Neg., Wass. Neg.	37	25	15.1
Micro. Neg., Kahn Pos., Wass. Pos.	6	6	3.6
Micro. Neg., Kahn Neg., Wass. Pos.	8	8	4.8
Micro. Neg., Kahn Pos., Wass. Neg.	10	6	3.6
Micro. Pos., Kahn Pos., Wass. A. C.	24	23	13.9
Micro. Neg., Kahn Pos., Wass. A. C.	1	1	0.6
Micro. Pos., Kahn Neg., Wass. A. C.	4	2	1.2
Micro. Neg., Kahn Neg., Wass. A. C.	3	1	0.6
Microscopic Test Positive	137	105	63.6
Tube Kahn Positive	105	83	50.3
Wassermann (Kolmer) Positive	22	22	13.3

In the class of patients coming to our hospital and out-patient clinic, the greater majority of which are negroes, it is very difficult to obtain a reliable history, so that those placed as clinically syphilis are from definite evidence or previous hospital history showing definite evidence.

It may be of interest to state that, in more than thirty thousand Wassermann tests made in Charleston, S. C., about 45 per cent positives are found among the negroes to 15 per cent positives among the whites. This, of course, represents repeated tests on cases under treatment as well as single tests.

It must be understood that the thirty-eight cases of disagreements excluded do not mean falsely positive tests, but that no definite history of syphilis was obtainable. Among the disagreements, the microscopic test was positive in 63.6 per cent. The Kahn was positive in 50.3 per cent, and only 13.3 per cent gave a positive Wassermann test; this latter was due to the fact that thirty-two of the disagreements were due to anticomplementary tests, twenty-seven of which have given an undoubted history of syphilis.

The results with the microscopic test in treated cases of syphilis have shown the same finding that the tube Kahn has, i.e., a greater number of positives are obtained by these methods than with the Wassermann test, showing the increased sensitiveness of the test.

From as careful a study as possible to obtain a reliable history, I could find no evidence that positive tests were obtained in cases definitely proved not to be syphilis. That is, none could be proved falsely positive.

I have used the microscopic precipitation test with cerebrospinal fluids, using the method of globulin concentration,[6] and have so far obtained practically parallel results with the tube Kahn and Wassermann tests. The number is still too small to make any decided statement in regard to its value with cerebrospinal fluids. However, for your information, results are here shown in Table III:

TABLE III

CEREBROSPINAL FLUIDS. COMPARATIVE MICROSCOPIC KAHN, TUBE KAHN, AND WASSERMANN TESTS

	NUMBER TESTS	
Agreement of all three	23	92%
Positive Agreements	3	All syphilis
Disagreements	2	8%
Micro. Pos., Kahn Neg., Wass. Pos.	1	Syphilis
Micro. Neg., Kahn Neg., Wass. Pos.	1	Syphilis

My thanks are here expressed to Dr. W. G. Gamble and Dr. S. Simons, Miss Eleanor Townsend, and Miss Lois Thompson for their valued assistance in this investigation.

CONCLUSIONS

The number of comparative results reported so far with the microscopic precipitation test are too few upon which to form any decided opinion. My results, however, compare favorably with those given by Kline and Young,[2] and I believe confirm their findings as follows:

1. The microscopic slide precipitation test is simpler to perform than the Kahn tube test, requires less material and time, and the results are more easily read.

2. The microscopic test is slightly more sensitive than either the Kahn or Wassermann tests and appears to be as specific.

3. The microscopic test appears to be as adaptable as the Kahn test to the globulin concentrated cerebrospinal fluids and checks favorably when compared with the Wassermann test.

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A COMPARISON OF THE KOLMER AND KAHN TESTS FOR SYPHILIS*

By C. E. Roderick, M.D., F. S. Salisbury, M.D., and C. G. Cates, Veterans' Bureau Hospital No. 100, Camp Custer, Mich.

IN THE past two or three years numerous articles have been written comparing the Kahn test with the so-called Wassermann test; this latter term being used to cover all systems employed or all pet methods used. We make this statement in view of the fact that until an attempt at some sort of standardization was made about three years ago, there were probably as many methods employed as there were serologists doing the test, and all with more or less success. However, when comparison is made between the Kahn and the complement-fixation test for syphilis, the method employed should always be given. It has been the experience of many laboratory workers throughout the country, as evidenced by statements made in regard to the Kolmer complement-fixation test at the annual meetings of the clinical pathologists, that the Kolmer test is perhaps superior to many of the methods used heretofore. So true is this that many of us have abandoned our pet methods in favor of the more delicate and reliable Kolmer method. In a number of the papers that used the term *Wassermann test*, the antigen has been mentioned, but in many more perhaps, only the term Wassermann test has been employed.

During the past eight months we have been interested in a comparison of the Kolmer complement-fixation test and the Kahn flocculation test in treated neurosyphilis at the Veterans' Bureau Hospital, No. 100, Camp Custer, Michigan. While the number of cases studied is small, we feel that our results are worth reporting at this time. Due to the relative frequency with which articles have appeared in the literature concerning the superior value of one or

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From Veterans' Bureau Hospital, No. 100.

the other of these tests, we decided to investigate each, relative to its merit as a guide to the treatment for syphilis. The number of cases selected is small, and upon many we did not have an entrance test with both methods due to the fact that the Veterans' Bureau Hospital No. 100 was established about two years ago, but the laboratory was not organized until about a year later. We shall show the results obtained by both the Kolmer and the Kahn method after one, two, three, four, five, and six courses of treatment. We are also running parallel with the Kahn and Kolmer tests the technic adopted by the Veterans' Bureau. The results of this comparison will be published at a later date.

The objection voiced, to the Kolmer as well as to many other ways of doing complement-fixation tests for syphilis, is that of the consumption of time, referring particularly to overnight incubation in the ice box. This, however, to our minds, is a more or less groundless objection, because time is not the most important factor in the serologic diagnosis of syphilis. In most instances we find syphilis has existed many days, months, or years before patients come to a doctor for advice Therefore, what great harm can be done in further delaying the report on the blood serum for twenty-four hours when we know at the time that we are perhaps going to have the most reliable result that can be obtained. In other words, accuracy should never be sacrificed for speed. In many instances the same physician who expects us to hurry through a twenty-four hour test in an hour or so for a disease like syphilis will sit by, in an acute infection like diphtheria, and wait for a report on the culture until the next morning.

Now the question constantly arises as to the advisability of substituting the Kahn test for the complement-fixation test for the diagnosis of syphilis and the control of the treatment. A very brief review of the opinions of several workers of the country who have been comparing the Kahn test with other methods might serve as an introduction to our experiences.

Owen found 93.8 per cent of 1,600 cases to agree, and he also found his diversions almost wholly in treated cases and considers the Kahn a valuable check in a small percentage of cases.

Giordano finds it valuable in emergency tests for donors for blood transfusions, but at the same time he explains the difficulty in reading a weakly positive reaction. He does not like to report a weak reaction unless it is checked by the complement-fixation test. In view of this statement it would seem to us that if it cannot be relied upon when it is not checked by the complement-fixation test it certainly should not be relied upon alone at all.

Kelly found them to agree in 94.45 per cent of 110 cases. He calls attention to increased flocculation when left in the ice box overnight. We have had this experience and have found that false positives may be obtained in this way.

Greenbaum found the Kahn to run positive in many cases after the Kolmer became negative and vice versa, and he advises that both methods be used. In our experience the Kahn becomes negative much sooner than the Kolmer.

Haven and Taylor conclude that the present value of this test is questionable as a Public Health laboratory method.

Lederer, in comparing it with the Veterans' Bureau Wassermann method, finds the Kahn test to be of value, and combining both tests simultaneously is the ideal diagnostic practice.

Becker concludes that the Kahn gives more uniform results in repeated examinations and is more difficult to change from positive to negative by treatment.

Anderson concludes that "the great reliability of the Kolmer method and the simplicity and apparent reliability of the Kahn precipitation test made this combination the best standard procedure for the diagnosis of syphilis."

Kilduffe finds that the Kahn test checks with the Wassermann reaction in from 80 to 90 per cent of cases, depending upon the care with which it is performed and the delicacy and reliability of the Wassermann technic with which it is compared and that false negatives and false positives occur with the Kahn test.

Houghton, in an article in the *Journal of the American Medical Association*, December 4, 1926, declares the Kahn test has removed the serum diagnosis of syphilis from empiricism and has placed it upon the basis of quantitative science, and in his summary calls attention to the fact that the Kahn test is more sensitive than the Wassermann test in treated cases. We feel that the former statement remains to be proved, and our experience with the following few cases of treated syphilis tells us that the second statement does not hold.

From the foregoing it seems that we have a few who advocate the Kahn test alone; more who advocate the Kolmer test alone, and a much larger number who emphatically advise the use of both tests as being the nearest to perfection.

Our figures in the short series we have ready at this time are as follows:

After one course of treatment

8 cases		
Kolmer positive in 4	50. %	
Kahn positive in 0	0. %	
Both positive in 3	37.5%	Agreement 50%
Both negative in 1	12.5%	

After two courses of treatment

15 cases		
Kolmer positive in 6	40. %	
Kahn positive in 2	13.4%	
Both positive in 4	26.6%	Agreement 46.6%
Both negative in 3	20. %	

After three courses of treatment

11 cases		
Kolmer positive in 4	36.3%	
Kahn positive in 1	0.9%	
Both positive in 4	36.4%	Agreement 54.5%
Both negative in 2	18.2%	

After four courses of treatment

10 cases

Kolmer positive in 6	60. %	Agreement in 40%
Kahn positive in 0	0. %	
Both positive in 2	20. %	
Both negative in 2	20. %	

After five courses of treatment

18 cases

Kolmer positive in 7	38.8%	Agreement in 55.4%
Kahn positive in 1	0.6%	
Both positive in 7	38.8%	
Both negative in 3	16.6%	

After six courses of treatment

14 cases

Kolmer positive in 5	35.7%	Agreement in 56.7%
Kahn positive in 1	7.0%	
Both positive in 5	35.7%	
Both negative in 3	21.0%	

After seven courses of treatment

13 cases

Kolmer positive in 5	38.5%	Agreement in 61.5%
Kahn positive in 0	0. %	
Both positive in 5	38.5%	
Both negative in 3	23. %	

Kolmer positive and Kahn negative in 37	or 41.5%	Agreement in 52.8%
Kahn positive and Kolmer negative in 5	or 5.6%	
Both positive in 30	or 33.7%	
Both negative in 17	or 19.1%	

In conclusion we wish to say that our position in this matter is neutral. We do not believe we are in a position to substitute the Kahn test for the Kolmer complement-fixation test at this time. In our minds the Kolmer test is more reliable and sensitive for the diagnosis of syphilis and as a prognostic indicator in treated cases. We believe, however, that the Kahn test is worthy of merit, and both tests should be used simultaneously until one or the other is eventually discredited.

DISCUSSION OF PAPERS BY KILDUFFE,* JOHNSON AND RODERICK, SALISBURY AND CATES

Dr. R. L. Kahn.—It is difficult in a short space of time to talk extensively on precipitation in syphilis. The problem is too big. I was interested in the conclusions of the speakers that while the Kahn test is reliable, it should be used with the Wassermann test. I am not recommending the abandonment of the Wassermann test, neither am I inviting anyone to use the Kahn test. Indeed, there is not a man or woman in this room or anywhere, whom I have ever asked to study this test. Those who have come to us at Lansing or have written me about the test, I have assisted gladly, but beyond that, whether one uses the Wassermann or the Kahn or both or any additional tests, the problem is outside my hands.

I recognize that it is not a simple matter for many of you to turn from complement-fixation tests to which you have devoted perhaps ten or fifteen years and turn to the problem

*See THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE, xii, July, 1927, p. 946.

of precipitation. On entering this room, I met a man who, about twelve years ago, began to point out the importance of titrating complement. A good many of you, like Dr. Ottenberg, gave much time and effort in developing improvements in the Wassermann test. It is not to be expected, therefore, that you would hastily abandon this work, but when the time comes and you decide to give the Kahn test a trial, I would urge you to do it correctly and not crowd it to the wall.

My experience has been, almost invariably, when the Wassermann and Kahn tests are used together that the latter does not get a fair chance. After giving much time and energy to the Wassermann test, laboratory workers find the additional test burdensome and do not carry it out correctly. Many of our visitors in the Michigan Department of Health come with a record of several years' experience with the Kahn test. Yet, when they see our work, they invariably admit that in many details they have not followed the correct technic. The thought that I wish to leave with you is not to please try the test, but when you finally decide to try it, to do it correctly. Obtain proper glassware and apparatus. See that your worker picks up the correct method in some dependable laboratory. Obtain some standardized antigen from a reliable biologic house or from the Michigan Health Department at Lansing. If you prepare your own antigen, let us help you in its standardization. Then it will take very little time, and you will be convinced of the practicability of the test, of its reliability as a diagnostic agent in syphilis and of its general superior qualities.

A word regarding antigen. Its standardization should be in the hands of expert serologists in central laboratories. I should like to suggest respectfully that possibly your organization might look into this antigen problem with a view of supplying the standardized product to your members who are removed from medical centers. If you could arrange with some biologic house, for example, to provide standard Kahn antigen to your members at some minimum cost, it would go a long way toward good results with the Kahn test by your group, and I would be glad to assist you in any way possible. I hardly need add that should your organization care to appoint a committee to study this test or should you designate certain laboratories to study it, I shall be glad to be of any assistance, should I be called upon for suggestions.

I should also like to bring to your attention the fact that the Kahn test embraces a series of procedures and in this regard opens up a new field for the clinical pathologist. Aside from the routine or diagnostic procedure, there is the quantitative procedure, which is of special value in specific therapy; the presumptive procedure, which is more sensitive than the routine test and is of value in special cases where this test is negative; the procedure with syphilitic fluid other than serum and the microprocedures; also the qualitative and quantitative procedures for spinal fluids. I venture to predict that several years hence, the public health laboratory will limit itself to the routine Kahn test, and the various additional procedures will be carried out and interpreted by the clinical pathologist.

Dr. Kline.—I would like to take up the discussion exactly where Dr. Kahn ended. There is great promise in the precipitation test for syphilis. Dr. Kahn has not only focused the attention of serologists of this country on this method of detecting syphilis, but he has also worked out additional principles for the test, especially demonstrating the importance of using concentrated antigen.

Our experience in this field began two and a half years ago when Dr. Kahn kindly assisted us in many ways to acquire the technic. I would like to express again my appreciation for his help. After doing a number of Kahn tube tests, we decided to try doing the tests by the open slide method. We had been using the open slide method of blood typing reported by Vincent during the war and had found it a satisfactory method for Widal's test and other bacterial agglutinations also. We did these tests on a microscopic slide with paraffin rings mounted on it to confine the ingredients. After determining proper sized rings and quantities of antigen and serum to be used, we found that the precipitation test for syphilis could also be done with accuracy by this simple method. At first we used the antigen advocated by Kahn, but finding it too sensitive to cold and to time, we tried other methods of preparation, and for the past several months we have been using an antigen that works as well at low temperatures as at ordinary room temperature and is a stable lipoidal

suspension in contrast to the unstable one of Kahn. One such indicator for syphilis, prepared March 30, still possesses its antigenic properties.

Concerning the mechanism of the precipitation test for syphilis, Weil's hypothesis that antibodies in syphilis are formed against body-cell substances liberated by the destructive activity of Spirochetae pallidae rather than by the spirochetes themselves will apply as well as it does to the Wassermann reaction. In fact Wassermann in 1921 claimed that the substance resulting from the union of the lipoidal antigen (lecithin) and its antibody should be called the Wassermann aggregate and that this aggregate is capable of doing two things: (1) binding complement, and (2) causing precipitation as in the Sachs and Georgi test. In the precipitation test for syphilis it seems that a hydrophilic colloid (lecithin) protects cholesterin (hydrophobic colloid) against precipitation by water unless some substance is present (in the patient's serum) that combines with the lecithin and interferes with its protective action when the cholesterin will precipitate out and give a positive reaction.

It is important to test out this theory, for if it is true and the simple indicator cholesterin can be used instead of the hemolytic system, then there is a possibility that a simple standard test for syphilis can be devised.

Dr. H. A. Heise.—As one of these poor, tired serologists, I would like to add something that is a little time-saving device in the use of the quantitative Wassermann. The Kahn test is completed, and then quantitative Wassermann tests are performed only on those serums which give a positive Kahn reaction, the other serums being run by the ordinary qualitative technic.

Dr. Robert A. Kilduffe.—The particular point in my paper upon which I wished to focus attention was the present propaganda for the *exclusive* use of the Kahn test. Dr. Kahn has told us that he has never by word or published utterance advocated its exclusive use. I feel, however, that the fact that it has been adopted as an exclusive test by the laboratories of which he is the serologist is an expression of opinion on this point and I cannot recall that Dr. Kahn has ever expressed any disapproval of its exclusive use or that he has ever suggested caution in so applying his test.

Dr. Kahn tells us that he has been written to by laboratories, having the exclusive use of his test in mind. It would be interesting to know what he has replied; if he has said that he considered this hasty or unwise, or if he simply acquiesced, expressed satisfaction, and offered a supply of antigen?

I consider it unfortunate, also, that this test has been consistently exploited and demonstrated almost exclusively before county medical societies to whose members the idea that all that is necessary is a pipette, a test tube, and a bottle of antigen is naturally attractive. The fact that, as I am told by a colleague, at a recent meeting of the American College of Physicians, two physicians expressed the intention of spending one hour in a laboratory to learn the Kahn test so as to use it in their offices is evidence that the impression is abroad that anyone may learn this test in a short time.

This is one of the impressions I desire to combat, and, therefore, I want to emphasize again that I do not by any means agree with those who disseminate it.

I think if this Society, representing as it does, a cross-section of laboratory workers, were to express its opinion as to the Kahn test, whether it should be considered an *additional* valuable method, or sufficient as an exclusive method in the serological diagnosis of syphilis, the opinion would carry great weight and would be an excellent thing for all concerned, not least among whom is the patient!

Dr. Robert A. Keilty.—I had the pleasure of starting with Kolmer in his original work. I believe I have heard Dr. Kahn's original presentation of his paper.

The future work of the Veterans' Bureau, which is now only beginning, will play an important part in this.

In fairness to the patient the diagnosis of syphilis is going to be very important, for instance, a diagnosis of syphilis alone; a clinical diagnosis of paresis being rather difficult.

I think we have spinal fluid absolutely conquered. In the case of the blood Wassermann we should have just as much evidence as we can have in that data. I agree with Dr. Kilduffe that all of them should be used.

Dr. Francis B. Johnson.—When we consider that the Wassermann test was introduced some twenty years ago, and realize that it took time and various modifications before its acceptance at its true value, we should give more consideration to the Kahn test and its modifications. These tests have comparatively only recently been introduced. While certain clinical pathologists and others realize, to their satisfaction, that it is of equal or greater value than the Wassermann, there are still many others that are not convinced, and until the majority are convinced, we have no right to give up the use of the Wassermann to some other test. The Wassermann repeatedly proves itself of value in cases with a negative Kahn and vice versa; therefore, we should do them all, for the best aid to diagnosis. I believe the Kahn is more sensitive than the Wassermann. If the one plus Kahn is compared to the one plus Wassermann, the Kahn is not as specific.

Dr. Kahn (closing).—One of the speakers brought up an interesting point. How is it, if I do not advocate the abandonment of the Wassermann test, that I, as "director of laboratories" of the Michigan Department of Health, rushed and abandoned the Wassermann test and made the Kahn test standard? In the first place, I do not "advocate" anything. I am not an advocate by nature or by training. My problem of late years has been to learn something about the precipitation phenomenon in syphilis, and with the accumulation of some facts, we gradually evolved what seems to be a practical and reliable method for the serum diagnosis of syphilis. This method was reported to Michigan physicians from July, 1922 to October, 1925, parallel with the Wassermann test. A total of 175,000 Kahn tests were thus reported. Dr. C. C. Young, the director of laboratories with the other administrative officers of the Michigan Department of Health, felt that the time had come to abandon the Wassermann, because the Kahn has consistently proved itself both more specific and more sensitive than the Wassermann test. It was a purely administrative problem and outside of my hands.

My interest in connection with outside workers is to help them do the Kahn test correctly. Beyond that my interest ends. Whether or not any one will continue to perform the Wassermann test after he has become expert with the Kahn test is an individual problem.

This I should like to leave with you, namely, that there is little question but that we are dealing in syphilis with one substance which can be detected either by precipitation or by complement fixation. Recently we have completed a series of studies, wherein we added the hemolytic system to completed Kahn tests. In other words after reading the precipitation results we added complement amboceptor and cells and read the complement-fixation results. In only 2 per cent of cases did we find disagreement between the two readings. What is well to remember is that the hemolytic system is merely an indicator and is in no way related to syphilis.

FATALITIES FOLLOWING THE USE OF ARSPHENAMINE*†

By Ernest Scott, M.D., and R. A. Moore, M.Sc., Columbus, Ohio

INTRODUCTION

SEVERE and undesired reactions following the administration of arsphenamine fortunately are not common. Their infrequency, however, should not cause one to overlook the fact that very dangerous and even fatal results may occur at the most unexpected moment.

Various forms of inorganic arsenic have for years been used in the treatment of disease. The concentration of these drugs, however necessary to destroy the disease-producing parasites, was in all cases harmful to the host. Therefore, the attempt of experimentation was to secure a compound of arsenic which would kill the parasite and yet be in such form or concentration that it would be relatively innocuous to the host. The first of these compounds was atoxyl or sodium arsanilate. This compound served its purpose but was still too toxic for general use. Ehrlich in his chemotherapeutic studies discovered para-dihydroxy-meta-diamino-arsenobenzene, or salvarsan. Later, by the addition of formaldehyde to the formula, he developed neosalvarsan. More recently Jacobs and Heidelberger[1] have prepared a derivative of atoxyl, tryparsamide. This substance was very thoroughly tested by Brown and Pearce[2] and is at the preset time recognized as a valuable drug, especially in the treatment of neurosyphilis. Within the last few years Loevenhart and his coworkers have prepared many new organic arsenicals and have tested their efficiency in syphilis and related conditions. The very general use of organic arsenicals proves that they have fulfilled the demands of the situation; namely, that of (a) being an efficient parasiticide, (b) being relatively harmless to the patient, and (c) being easy and safe of administration. There are, however, a certain few who possess an idiosynerasy and in whom more or less severe reactions occur. Such reactions vary widely in their form and intensity but are uniformly unexpected and undesirable.

CASE HISTORY

C. A., aged sixteen, white, female, was admitted to the venereal clinic four weeks previous to death. She had been given three injections of neoarsphenamine. On May 29, 1926, an injection of 0.6 gm. of neoarsphenamine was given with no immediate untoward effects. Six other patients were given injections of the same lot of drug and experienced no ill effects. On May 31, or two days after the injection, the patient complained of a slight headache and malaise. This malaise and headache became very severe in the evening, and early on the morning of the next day she became comatose. There was no nausea or vomiting at any time. On June 1, or three days after the injections, another one of the patients became ill with the same symptoms. This latter patient became comatose the same after-

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†From the Department of Pathology, Ohio State University.

noon. Both patients presented the same clinical symptoms; namely, headache, malaise, and coma; both received neoarsphenamine intravenously and were taking iodides by mouth, but no mercury had been used in either case. In the terminal twenty-four hours of life in both cases there was a complete anuria. The first patient died on June 2 and the second on June 1.

Through the kindness of Dr. C. P. Robbins, of Columbus, Ohio, we were able to perform an autopsy upon the first patient with the following findings: Upon opening the body, an inspection of the abdominal cavity showed that the subcutaneous fat was normal in amount. The omentum was large, and contained a considerable quantity of fat. The liver was slightly below the costal margin but not noticeably enlarged. The surface of the right lobe was darker in color than the remainder of the liver and had a somewhat dry, wrinkled appearance. The appendix was retrocecal, about 4 inches long, and slightly larger in diameter than normal; it showed no evidence of recent inflammation. Within the pelvis the peritoneum surrounding the uterus was covered by a bloody fluid; this fluid was easily removed and the peritoneum itself was of normal color. The urinary bladder was contracted and empty. In the left side of the peritoneal cavity in the region of the spleen, there was a large amount (4 or 5 ounces) of bloody fluid, similar to that found in the pelvis. The right side of the peritoneal cavity was entirely dry and normal in its appearance. There was no evidence of peritonitis or inflammatory exudate in any portion of the peritoneal cavity.

Thoracic Cavity.—The anterior margin of the left lung was prominent and apparently emphysematous. In the left pleural cavity there was a large quantity of bloody fluid similar to that seen in the peritoneal cavity; this fluid was somewhat thicker than the usual pleural exudate and was of a somewhat slimy mucoid character. On removal there was found to be approximately one quart of this fluid. The right pleural cavity was perfectly normal in its appearance. The thymus gland was distinguishable. The anterior mediastinal glands were not visible. The pericardial sac was normal in appearance and contained the usual amount of clear fluid. The left lung was of normal size. The pleura, especially of the posterior portion, was a deep red color but did not show evidence of inflammatory change or exudate. The lung was crepitant throughout, although the lower lobe was slightly firmer in texture. There were no areas of consolidation. On section the lower lobe was edematous and was of a deep red color. Crepitation in this portion was somewhat limited. The vessels at the root of the lung were free of clot. The bronchi were normal in appearance with a slight congestion of the mucous membrane. The right lung was crepitant throughout, although the posterior portions were slightly firmer and of a darker color. The vessels were free and unobstructed. The bronchi contained a small amount of frothy mucus, and the mucous membrane was congested. Section through this lung showed an edematous condition of the posterior portions of the lower lobe. The heart was of normal size and appearance. On opening the heart the cavities seemed to be of usual size and appearance, and the valves were all normal in appearance. The myocardium was of the usual color and consistency. The aorta showed a very slight atheromatous change at its base and also in the transverse arch and in the abdominal portion.

Organs of the Abdominal Cavity.—In attempting to remove the spleen, the bloody fluid surrounding it was removed and an inspection of this region revealed the fact that a large portion of the cardiac end of the stomach was absent and that the diaphragm immediately to the left of the vertebral column was also missing. Examination of these organs showed that the stomach wall and diaphragm had both disappeared because of necrosis and sloughing and that the fluid which surrounded the spleen and filled the left pleural cavity consisted of stomach contents mixed with blood. The spleen itself was of normal size; its capsule was of a dark reddish color. On section the spleen was darker at its lower lobe. There was no evidence of necrosis, however, either about the capsule or in the spleen substance. The tail of the pancreas was darker in color and somewhat softer than normal, this portion being surrounded by the fluid in the vicinity of the spleen. Other portions of the pancreas were normal in their appearance and consistency. The adrenals were normal in size and appearance. The kidneys were similar in appearance; their capsules stripped very easily. The surface of the cortex was normal in color and smooth. On section the markings were distinct and the pelves were normal. The lower pole of the

left kidney was somewhat darker in color than the remaining portion. The ureters were both normal in appearance. On inspection it was found that a large portion of the posterior surface of the cardiac end of the stomach had sloughed away; the margins of the slough were very necrotic and thin. The stomach contents had escaped through this sloughed portion into the peritoneal cavity, and a slough of the left side of the diaphragm had allowed the stomach contents to pass into the left pleural cavity. By measurement, there was approximately 6 inches of the stomach wall missing, and the opening into the diaphragm measured 3.5 inches. Upon opening the stomach there were no signs of ulceration or of local inflammatory change. The mucosa of the pyloric portion was somewhat congested but otherwise of normal appearance. The posterior surface of the stomach wall was covered with tenacious mucus. The intestines were distended and were normal in their appearance. Examination of the mucous membrane of the entire small intestine and colon failed to reveal any areas of marked congestion or hemorrhage. The uterus was of normal size and consistency. The right fallopian tube was somewhat enlarged and was thin-walled. On section there was seen a bloody serous fluid; there was no evidence of pus. There was a small single cyst on the right ovary measuring one-half inch in diameter. The left fallopian tube was also somewhat larger than normal; its walls were thin; the fimbriae were shortened. The ovary measured 2 inches in diameter, and contained several small follicular cysts. On opening the uterus it was seen to contain a small amount of blood-tinged mucus in the fundus. Otherwise it was normal in appearance. The liver was of usual size and consistency. On cut-section the tissues appeared somewhat darker than normal, but the liver contained only a small amount of blood. The gall bladder was normal in size and moderately filled with bile, but contained no calculi.

Examination of the bony portions of the trunk, including the vertebral column, the ribs, and the bones of the pelvis, showed no evidence of pathologic change.

The Head.—The scalp, calvarium, and the dura mater were all normal in appearance. The pia mater over the surfaces of the hemispheres was much congested, the veins and capillaries being very distinct. The brain was of normal size. The convolutions were full, and the sulci somewhat inconspicuous. There was no evidence of meningitis or of inflammatory exudate. After removal of the brain, examination of the vessels at its base showed no evidence of vascular disease. On the inferior surface of the right temporal lobe there were a few very indefinite granules resembling miliary tubercles. On section into the hemispheres of the brain, small, dark, apparently necrotic areas were encountered in the roof of each lateral ventricle. Examination of the bones at the base of the skull showed no evidence of pathologic changes. Examination of the brain made in the laboratory revealed that there were other softened necrotic areas involving the pons, the crura cerebri, and the posterior portion of the optic thalmus; these areas resembled those seen at the time of the postmortem, involving the tissues of the wall of the lateral ventricles. The most extensive hemorrhage was seen in the pons.

The anatomic diagnosis was that of (1) congestion, capillary hemmorhage, hyaline thrombosis and edema of the brain; (2) postmortem autolysis of the stomach wall and diaphragm; (3) edema of the posterior portions of the lungs, and (4) acute tubular nephritis.

The microscopic examination of sections taken from the degenerative region in the brain showed that the pathologic changes consisted of very intense congestion with small hemorrhages and edema. These changes were most pronounced in the pons but were very definite in the other involved portions of the brain. The walls of very many of the congested vessels showed an infiltration with lymphocytes and polynuclear leucocytes. This infiltration apparently involved the vessel wall and was not within the perivascular lymph spaces as seen in the vascular changes of encephalitis lethargica and in poliomyelitis. Sections from the kidney showed a very pronounced tubular nephritis involving the epithelium of all the convoluted tubules and Henle's loop. Sections of the stomach taken near the margin of the slough showed a gradually increasing degeneration, and autolysis of the cells at the edge of the necrotic area was approached. Sections from the remaining organs showed no definite pathologic change.

Chemical examination of the liver revealed the presence of small quantities of arsenic. Toxicity tests on ampules of neosalvarsan from the same lot used, showed that they conform to the recognized standards.

CLASSIFICATION OF REACTIONS

The British Medical Research Council[3] in their report sum up the reactions which may follow the administration of organic arsenicals as follows:

"1. Immediate reactions consisting of diarrhea, vomiting, pyrexia, and headache. Under this group are also classed the so-called vasomotor phenomena and the anaphylactoid and nitritoid crises.

"2. Effects on the nervous system. The most important of these is encephalitis hemorrhagica.

"3. Effects on the liver. These are clinically grouped into:

"a. Early jaundice—coming on in a few days, usually mild and evanescent but rarely more severe and persistent.

"b. Late jaundice—not earlier than several weeks of a more severe and prolonged type.

"c. Acute yellow atrophy of the liver, supervening on late jaundice.

"4. Exfoliative dermatitis and slighter skin reactions. The former may be complicated with bronchopneumonia or septicemia and end fatally.

"5. Various rare lesions, such as acute hemorrhagic nephritis, ulcerative enteritis, and aplastic anemia.

"In addition to these complications, there are a number of other conditions which were previously believed to be due to relapses of the disease or the sudden liberation of toxins from the killed treponema. In this group are the so-called 'Herxheimer reaction,' deafness, cranial nerve palsies, and other forms of 'neurorecurrence.'"

INCIDENCE

Statistics upon the explainable fatalities following the use of organic arsenicals are unsatisfactory. Phelps[4] cites Lake, of the United States Public Health Service, as stating that he had collected thirteen deaths following the use of arsphenamine and the same number following the use of neoarsphenamine in the United States. In the United States Navy from 1919 to 1923, out of 15,170 cases of syphilis treated with an unknown number of injections, there were fourteen deaths. Phelps cites German statistics as stating that there was one death for every 3,788 cases treated with 10,984 injections. The British Medical Research Council report two sets of statistics, Hospital A, one death in 1,629 cases with 13,000 injections and Hospital B, one death in 375 cases treated.

From these statistics, it becomes apparent that there is one unexplainable fatality in each 5,000 to 10,000 injections. Phelps, however, states that with neosalvarsan there should not be more than one fatality in every 40,000 injections.

THEORIES OF ETIOLOGY

From the papers of Ehrlich, Bernard, Danysz, Warthin and Schamberg, the theories of the etiology of these fatalities may be summarized as follows:

1. Due to previous or concurrent kidney disease, there is a faulty elimination of the drug, with a resultant arsenic poisoning.

2. That it is essentially a Herxheimer reaction due to the sudden liberation of a large amount of endotoxin from the killed treponema.

3. That it is due to the destructive and dilating action of arsphenamine on the blood vessels which is not counteracted by adrenalin, which is deficient perhaps because of arsenical destruction of the adrenals.

4. That the injection of arsphenamine into the blood stream causes a precipitation of the blood proteins and consequent multiple emboli.

Pearce and Brown[5] report, that in their experiences following the use of salvarsan, hemorrhage and congestion of the kidney, congestion, hemorrhage and necrosis with degeneration and a reduction of the chromaffin content of the adrenals are found. Kolmer and Luck[6] found that the injection

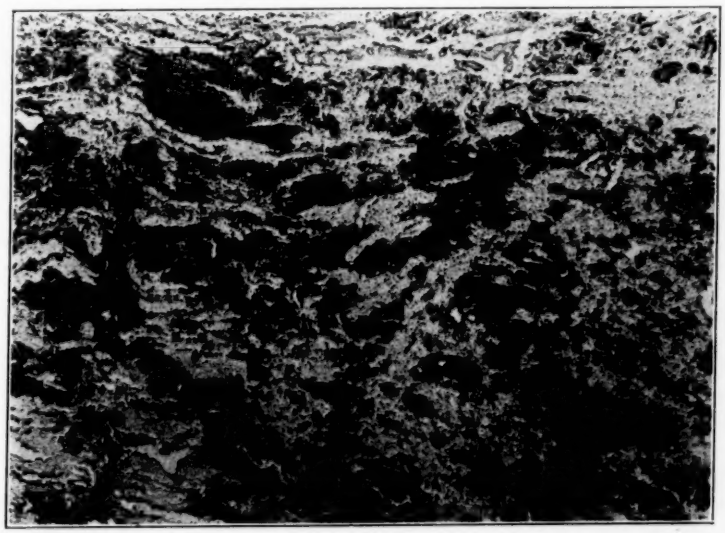

Fig. 1.—Low power of area of multiple capillary hemorrhage in the pons.

of acid solutions of arsphenamine produced widespread vascular injury. Single overdoses of the disodium salt produced severe vascular and tissue alterations, particularly in the liver, kidney, suprarenals, and spleen. Repeated therapeutic doses produced no effect. The same results, except of a milder form, were secured with neosalvarsan. Jackson and Smith,[7] from their pharmacologic experiments with salvarsan and neosalvarsan, came to the following conclusions: (1) slow injections in dogs produce no effect; (2) if the rate of injection or the concentration of the drug is increased, there is a progressive increase in pulmonary blood pressure and a slow decrease in the systemic blood pressure; (3) large doses may precipitate delirium cordis.

PATHOLOGY

Because of the diversity of types of reaction, there is no one set of pathologic findings which is found in all cases.

In those cases in which the prominent findings are in the brain, the term "hemorrhagic encephalitis" has been applied. Grossly the brain from such a case is congested and markedly edematous. Throughout the brain substance the vessels bleed easily from cut section. In the lateral wall and roof of the lateral ventricles are many punctate hemorrhages which may fuse into one hemorrhagic mass. In addition to this hemorrhagic ependymitis there are usually many small hemorrhages in the substance of the pons which if extensive enough may cause the pons to appear as one large hemorrhagic mass. On microscopic examination several findings are of importance. First, the brain substance is not degenerated, and it is readily seen that the large gross hemorrhage is made up of many small hemorrhagic areas. In many of the smaller blood vessels the lumen is occluded by a

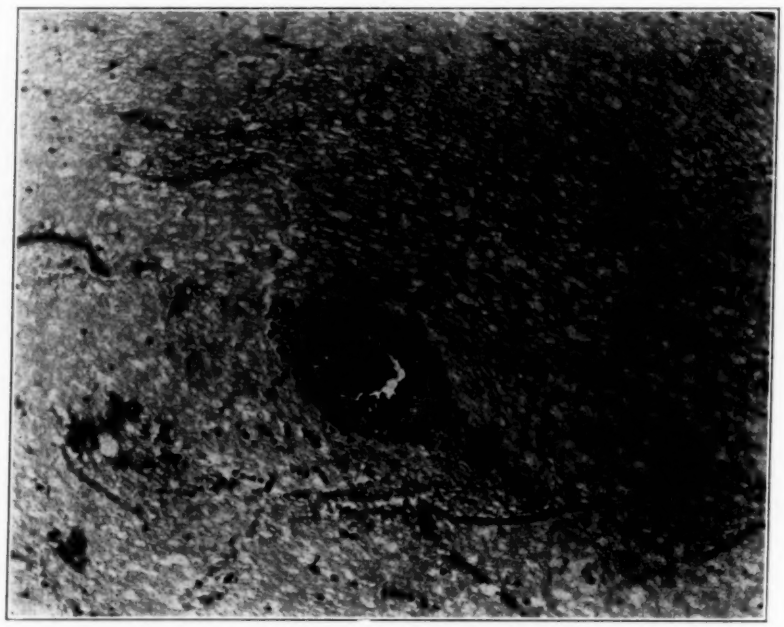

Fig. 2.—Section of pons showing perivascular infiltration of cells and hyaline thrombosis of smaller vessels.

hyaline thrombus. This hyaline thrombosis is not found in any other organ except the brain. In the present case the arterioles are surrounded by a collar of infiltrated wandering cells, mostly lymphocytes. In contrast to the accumulation of cells in the spaces of Virchow-Robin in other diseases, the infiltration in this case appears to be in the actual wall of the vessel and not in the perivascular lymphatics.

The pathologic changes in the liver in which jaundice is the prominent clinical symptom vary from a cloudy swelling to typical acute yellow atrophy depending upon the severity of the reaction. The relation of this hepatic damage to the injection of arsphenamine involves many questions. Is the hepatic damage due to syphilis, and its occurrence at this time only a coincidence? The weight of opinion would seem to be that the arsphenamine

itself causes the hepatic degeneration. The results of Wallis that injection of arsphenamine causes a slight to moderate hepatic insufficiency in cases where there are no clinical symptoms of liver disorder, would tend to confirm this opinion.

In most cases some pathologic condition of the kidney is reported. Various terms have been applied, such as "tubular nephritis," "acute hemorrhagic nephritis," etc., so that from a review of the reported cases it would appear that no definite and single change is characteristic. In the present case the nephritis was strictly tubular, involving all the tubules of the kidney and consisting of cloudy swelling and necrosis of the epithelium. As in the case of the liver changes, the etiology of the kidney pathology is obscure. Syphilis itself is capable of producing a mild nephritis and further simultaneous treatment with mercury must be considered. The ability of mercury to produce a nephritis is undoubted and in those fatal cases in which mercury and salvarsan have both been administered, the rôle of salvarsan in producing the nephritis must be carefully evaluated. In cases like the present one where clinically there was an anuria, and at autopsy an acute tubular nephritis, with salvarsan the only drug given, there can be little doubt that the salvarsan produced the nephritis.

The relation of salvarsan to degeneration of the adrenals is of interest. Experimentally it has been observed by Pearce and Brown and was observed in the present human case.

Other rarer pathologic lesions, such as ulcerative enteritis and destruction of bone marrow,[8] have been reported in isolated cases.

DISCUSSION

In looking over the results of the postmortem findings on human beings and the results of the experiments on animals, we find that there is a great similarity, indicating that by experiments on animals we may duplicate the conditions found in these unexplainable deaths from arsphenamine. Further, there is a striking resemblance of the pathologic findings in both the above two groups and the pathologic findings in an ordinary case of inorganic arsenic poisoning. The essential pathologic findings in all three classes of cases being:

1. A destructive action on the endothelium resulting in congestion and hemorrhage in most of the organs of the body.

2. The poor circulation resulting in edema of the brain and lungs in particular.

3. A nephritis, usually of the tubular type, but under certain circumstances taking on also a glomerular type.

4. The finding of infarcted and necrotic areas in the various viscera in some cases.

5. Necrosis of the liver, and sometimes acute yellow atrophy.

From a critical survey of the pharmacology, proposed theories of pathogenesis, and the observed postmortem findings, and in view of the facts brought out above that arsenic poisoning, experimental arsphenamine poisoning, and the fatalities with arsphenamine in human practice are very similar

in their pathology, we wish to cite what appears to us as the most likely theory in regard to the pathogenesis, namely,

1. That the immediate reaction, sometimes ending fatally, is due to the specific action of the arsphenamine molecule in its action by dilating the blood vessels of the body, resulting in some symptoms due to the local effects of congestion and stasis and others due to the same factors operating through the central nervous system centers. On this basis we may explain the "nitritoid crises," so-called because of their resemblance to the action of the nitrites in dilating the blood vessels, the headache due to a congestion of the brain, the vomiting and nausea due in part to the congestion of the stomach and to poor circulation in the cerebral centers, and all the so-called shock effects and vasomotor phenomena.

2. That the essential nature of the delayed reactions is that of an acute or subacute arsenic poisoning due to faulty elimination of the arsphenamine molecule or the arsenic set free from it and the consequent liberation of the arsenic in inorganic form, which not being eliminated is retained and acts as a poison. The retained arsphenamine may act as a vasodilator and enhance the action of the arsenic as a capillary poison.

3. Any part that adrenalin may play in the reaction, other than counteracting the vasodilatation, is very doubtful in our mind.

4. As to the embolic theory, although we cannot dispute it, at the same time we do not believe that it plays any essential part in the resulting pathology.

In summary we may then say that the delayed arsphenamine reaction is nothing more than an arsenic poisoning due to the liberation of poisonous trivalent inorganic arsenic from the faultily eliminated arsphenamine.

PROPHYLAXIS AND TREATMENT

It is not the purpose of this paper to enter into the field of the therapeusis of the organic arsenicals, but it is within our scope to point out facts which, gleaned from experimental pathology and human pathology, might be of value in the prevention of these fatalities:

1. First the examination of the kidney is of prime importance since practically all the cases at postmortem show a nephritis. Therefore, it is of the greatest importance that in the administration of organic arsenicals that we do not give them to a patient whose kidneys are already having difficulty in eliminating the ordinary waste products of metabolism and do not overload them with the elimination of an inorganic metallic poison.

2. Since it is known that mercury will act as a causative agent in producing nephritis, the use of any considerable quantity of mercury should not be practiced when the administration of arsenicals is occurring at the same time or within a short time. In other words, in the use of these two inorganic metallic poisons, we should use caution and occasionally give the patient a rest, so he may recover a normal metabolism.

3. That the best care and technic which it is possible to use be practiced in the administration of the drugs; that is sterile, freshly distilled water, com-

plete neutralization if arsphenamine is used, sufficient volume, not too fast injection, and not too heavy dosage at first or if the patient shows any intolerance for the drug.

As to the treatment of these reactions, there is not a great deal of material or deduction of facts from human practice. It has been suggested by several authors that adrenalin given to the desired effect be used, and several authors conclude that it is a specific. Jackson and Smith[7] suggest the use of tyramine which differs in no great respect from adrenalin in its pharmacologic action.

It has been pointed out by Jackson and Smith, and rightly, that in the vasomotor and circulatory disturbance that follows the use of arsphenamine the heart is in a condition of instability and may go into delirium cordis. We know that adrenalin and the other amines may under certain circumstances produce delirium cordis; therefore, in the use of these drugs, there is a certain danger, which is unavoidable. This possibility is to be kept in mind but is not to be considered as a contraindication since the amines are the only drugs we have that offer any chance of benefit.

Since arsenic is found in the tissues of these patients and since arsenic poisoning is probably a big factor in these fatalities, there is every reason for carrying out the usual procedures to promote the elimination of the poison by any means that the condition of the patient will tolerate.

CONCLUSION

In conclusion the following statement made in the British Report seems fitting: "There are, and there always will be, certain exceptional individuals who will react to the drug (arsphenamine) more severely than others and in whom a dose, or series of doses, harmless to the average individual, may set up dangerous or even fatal complication."

Concerning the question of whether these fatalities are to be taken as evidence for the discontinuance of arsphenamine therapy, the committee further states, "The committee has no doubt that, in the interests of the patient himself, no less than in those of the community, the choice should be in favor of arsenobenzol treatment. We believe that the very small number of deaths which are unavoidably due to this treatment are immeasurably outweighed by the deaths and disabilities which would arise if the older methods of treatment of syphilis were alone practiced. At the same time the facts which have been brought out in this report no less strongly emphasize the importance of the most scrupulous care in the administration of a drug which of necessity is employed in doses not far removed from the danger line."

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LABORATORY METHODS

A CYTOLOGIC KEY TO THE DIAGNOSIS AND PROGNOSIS OF NEOPLASMS*

By Wm. Carpenter MacCarty, M.D., Rochester, Minn.

EVERY pathologist has had some kind of an unwritten plan for grouping specimens upon which he feels he must pass clinical judgment. Usually he divides them into the three following divisions, each with two subdivisions: inflammatory (acute and chronic), neoplasms (benign and malignant), and questionable (inflammatory or neoplastic). The inflammatory group is characterized by one or more of the following phenomena: congestion, edema, necrosis, leucocytic, lymphocytic and endotheliocytic infiltration, fibroblastic and fibrocytic proliferation, hyalinization, and such cytologic changes as granular degeneration, fatty degeneration, vacuolization, pyknosis, and the presence of occasional giant cells. Certain of these differentiate the condition into acute and chronic inflammation although there is usually no sharp line of demarcation. The neoplastic group is characterized by the presence of a mass or masses of cells which do not have the exact histologic arrangement of normal tissues but seem to be displacing normal tissues by expansion or invasion. If the cells are regular in size and shape, are encapsulated, and have the morphology of normal adult types of cells, the condition is benign. If, on the contrary, the cells have not the low power arrangement of normal adult cells, are irregular in shape and size, and contain asymmetrical mitotic figures, are hyperchromatic, and replace normal tissues by invasion and infiltration, and especially if the mass is nonencapsulated, the condition is malignant. There seems to be, however, no sharp line of demarcation between these two, in some instances. The third or doubtful group is characterized by a combination of the characteristics of Groups I and II and as such presents the greatest differential diagnostic difficulties for all histopathologists.

These statements will call to the minds of many histopathologists the difficulties which they have experienced in conscientiously endeavoring to render clinical service in diagnosis and prognosis for the direction of therapeutic procedures. When the pathologist fails to make a diagnosis, the clinician must of necessity make a guess based on his own experience. When the clinician follows the pathologist's mistaken diagnosis and prognosis, and directs his therapy accordingly, the pathologist, clinician, and patient usually suffer. Mistakes on the part of both pathologist and clinician are common and are due generally to several facts:

1. The clinical history of disease is not always differential unless the conditions are in the late or extreme stages. Any patient may have inflammatory and neoplastic conditions in the same lesion.

*Read before the Sixth Annual Convention of the American Society of Clinical Pathologists, Washington, D. C., May 13, 14, and 16, 1927.

2. The gross appearances of disease are not differential in over 80 per cent of cases, and then only in advanced or extreme stages of the disease.

3. Many diseases are internal and are, therefore, not macroscopically visible.

4. There is no reliable serum, chemical, physical, or skin reaction which speaks for or against malignant neoplastic disease.

5. Neoplastic diseases are coming to the profession as smaller and less visible lesions (Fig. 1).

6. Detailed positive clinical diagnoses are becoming relatively less frequent.

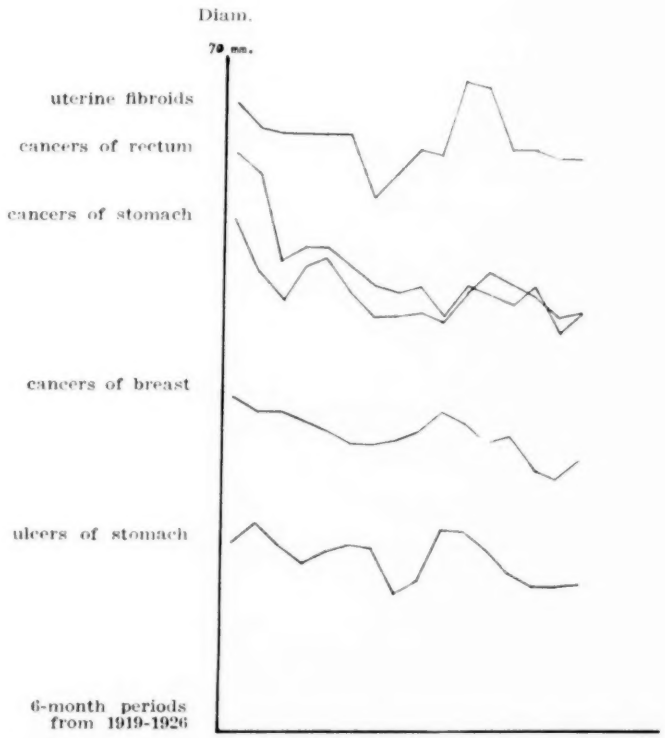

Fig. 1.—Diagram showing the decrease in size of cancers as the public and profession become educated to the necessity of early diagnosis.

To these facts must be added lack of cooperation on the part of pathologists and clinicians and lack of interest in tissue pathology and clinical medicine on the part of most pathologists. In general it may be said that tissue pathology, in direct relation to the practice of medicine, has been at a low ebb for the past twenty years and is just about to rise again. Part of the low ebb has been due to a failure on the part of pathologists to advance their own knowledge of human cytology. Pathologic histology has remained almost as it was given by Müller and Virchow fifty years ago; there have been some minor advances, but these have been along the lines of histology rather than cytology. There are at least three reasons for this: the limitation of

detailed cytologic study of postmortem fixed and embedded material, the pathologist's diversion into bacteriology, serology, immunology, and chemical pathology, and the lack of study of living and perfectly fresh human material.

In the last twenty years a new opportunity for study has arisen through operative surgery. A new material has been presented, and few have taken the opportunity of making new observations and checking these against in-

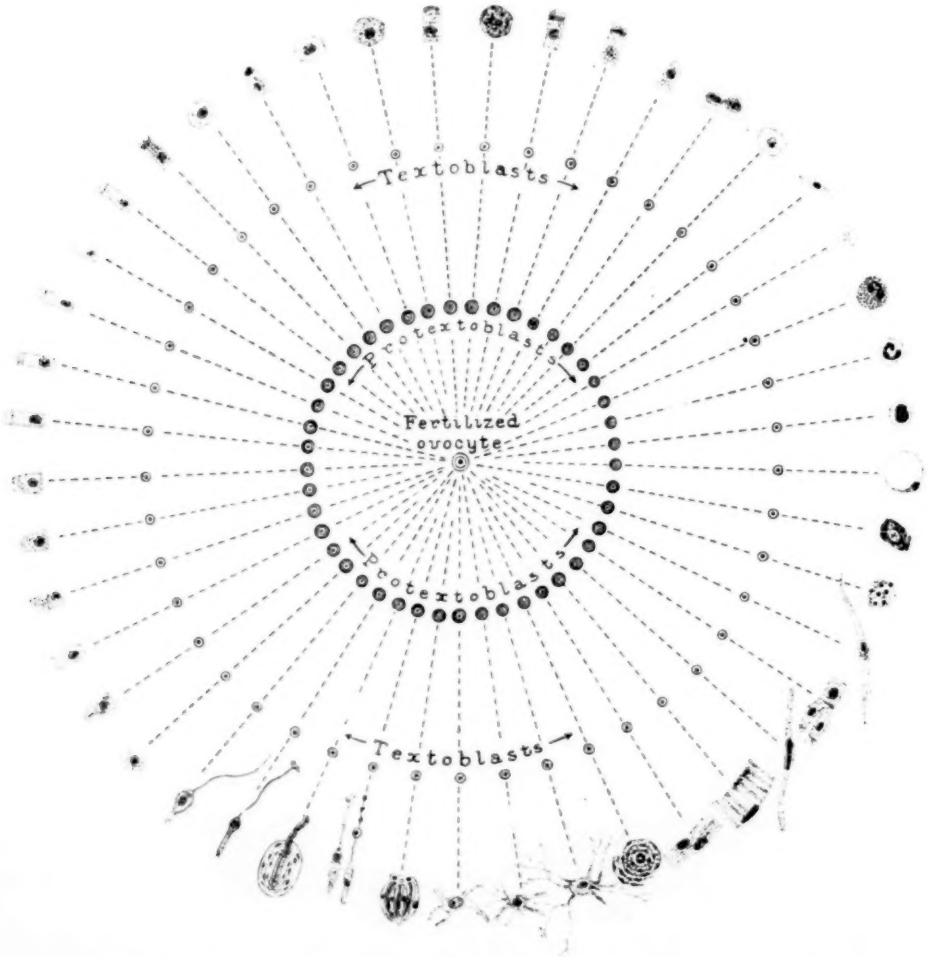

Fig. 2.—Diagram showing the evolution of at least forty-five different types of cells which constitute the tissues of the human body. No attempt is made to picture the exact morphology of each type although some of their characteristics are suggested.

timate clinical experience. So far as I know, the Mayo Clinic is the only place where this new opportunity has been given extensive practical attention over a long period of years. During the last twenty years more than 337,000 operations have been performed, and every operation was done with the pathologist present; all facts—the patient, history, visualization of the anatomic structural relationships, and the removed tissues—were available

and used by the pathologist in order to obtain data for correlation with macroscopic and microscopic findings.

In 1905 Wilson described a method of frozen sections. It was intended primarily for diagnostic histologic purposes, but has been developed into a method for cytologic study with the highest powers of the microscope. Cells soon became the object of interest and study. At first, confirmation or refutation of clinical diagnosis was uppermost in the minds of both clinicians and pathologists. Later it became apparent that malignant conditions were discovered which were not suggested by, or even suspected in, the clinical

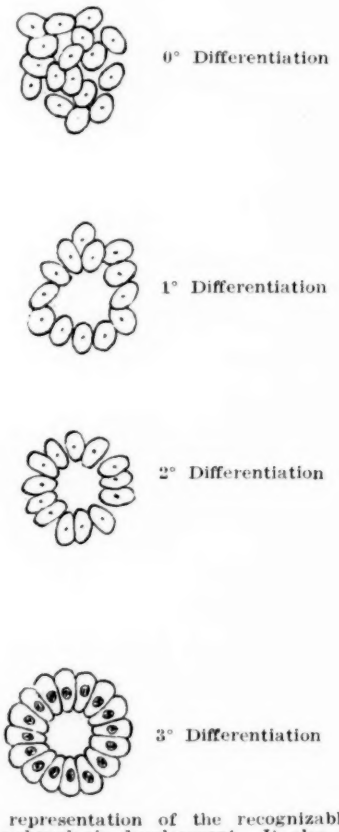

Fig. 3.—Diagrammatic representation of the recognizable stages of differentiation of a gland unit during normal embryologic development. It also represents the stages of differentiation in neoplastic conditions.

history or gross appearances. As progress was made, it was found that 57 per cent of all surgical cases presented material, and that 12.6 per cent of all cases required special pathologic consultation during operation, and that in 2.2 per cent of all surgical cases the diagnosis, prognosis, and therapeutic procedures were changed. In 0.5 per cent of all surgical cases, an unsuspected malignancy was found. The necessity for, and the value of, rapid and immediate diagnosis during operations brought about study and systematization of facts and the establishment of a plan for diagnosis and prognosis, only the cytologic part of which will be suggested at this time. It is

based upon the following facts: the human body is an organism composed of groups of cells of at least forty-five different types, all of which have evolved from a fertilized human ovum (Fig. 2). In this evolution, fertilization, segmentation, differentiation, and specialization occur. Tissue differentiation, in the human body, occurs in such a manner that it may be divided into three recognizable stages (Fig. 3): first, the establishment of the general alignment of the cells which is seen in the normal arrangement of adult tissue, the cells themselves remaining undifferentiated; second, the establishment of cellular polarity, such as is seen in fully differentiated tissue; and, third, the establishment of adult morphology of the cells. In the condition

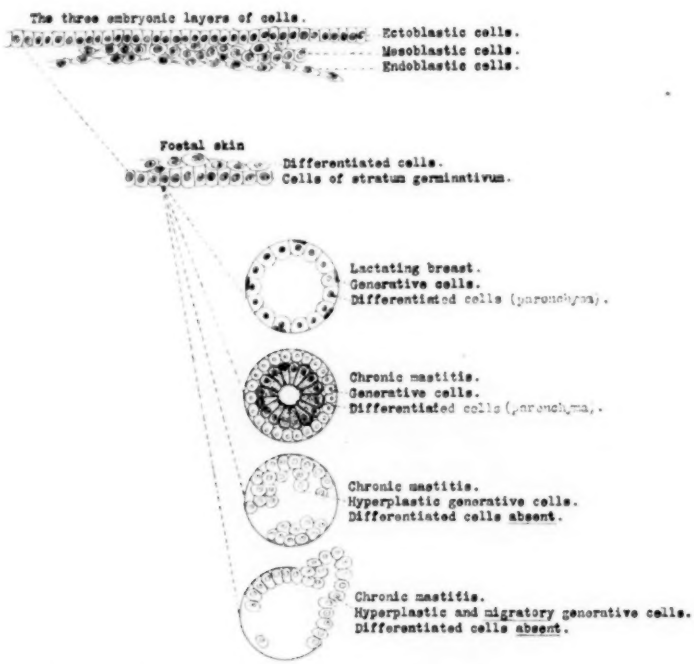

Fig. 4.—Diagrammatic representation of the evolution of a mammary acinus with its two types of cells (adenoblasts and adenocytes). The lower three diagrams show the three changes which are found in chronic mastitis. They represent hypertrophy, hyperplasia, and migration of adenoblasts. The migratory stage is malignant and the hyperplastic stage is potentially malignant.

of no differentiation, and the first and second stages, just described, the cells bear no morphologic resemblance to their adult forms.

During differentiation and specialization, nature provides for the phenomena of destruction and regeneration,—one a cause and the other an effect or reaction. Destruction of any tissue may be caused by many things. Regeneration of adult tissues occurs in two ways in the human body: directly (regeneration of adult cells from adult cells), and indirectly (regeneration from reserve cells). The malignant cell or the cell which has been called a "cancer cell," in which we are clinically especially interested, is evolved from the reserve cells although it may be derived directly from a cell which is normally regenerated directly. The study of the malignant cell, its be-

havior and the natural defensive reactions of the organism to its abnormal proliferation and invasion of the rest of the body, constitutes a problem of greatest practical economic importance; it is the key to both diagnosis and prognosis.

Perhaps the simplest way to present the facts as they have been seen in the biologic and cytologic study of neoplasms, is for us to confine our attention to principles in one organ. In Fig. 4 one sees, diagrammatically illustrated, the evolution of the milk-producing cells of the mammary gland. From the ectoblastic layer of the three layer stage of development of the

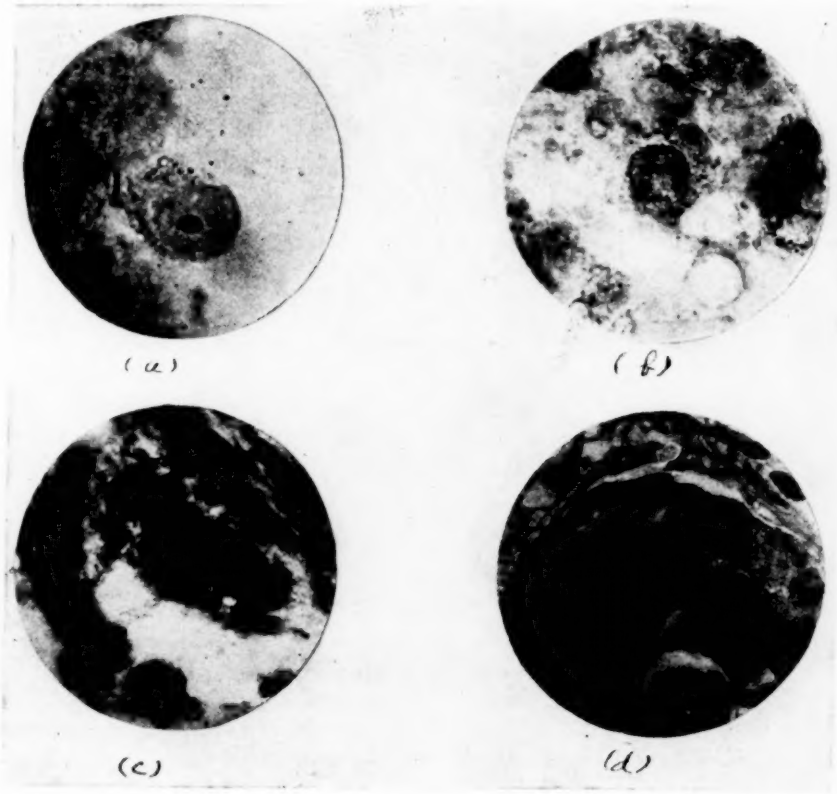

Fig. 5.—Photographs of malignant cells in a living state (*a, b, c*) and also in a fixed but not embedded state (*d*). These cells have positive diagnostic morphology.

embryo, the cells of the stratum germinativum of the fetal skin arise; from them, arise the fetal squamous cells by differentiation. Also from them, by multiplication, down growth into the subepithelial tissues, and differentiation, the lining cells of the mammary tubules and acini arise. It may be seen that there are two layers of cells in each normal mammary acinus and tubule. The cells lying adjacent to the lumen are secretory (adenocytes), and those lying next to the stroma are the reserve cells (adenoblasts). In chronic mastitis some one or more unknown things or conditions destroy the secretory cells; the reserve cells become hypertrophic or enlarged. This is a common picture in chronic mastitis with or without the presence of can-

cer. In some of the acini the reserve cells not only become hypertrophic, under the condition of destruction of the secretory cells, but also become hyperplastic; they increase in number and partially or completely fill the acinus. In this condition the cells are undifferentiated; they have larger nucleoli and nuclei than do adult or differentiated cells. The undifferentiated cells are frequently indistinguishable from those of cancer despite the fact that they are still within the confines of the acinus. In some acini, in some chronic mastitides in which this proliferative condition exists, one finds the line of demarcation between acinus and stroma destroyed by the migration

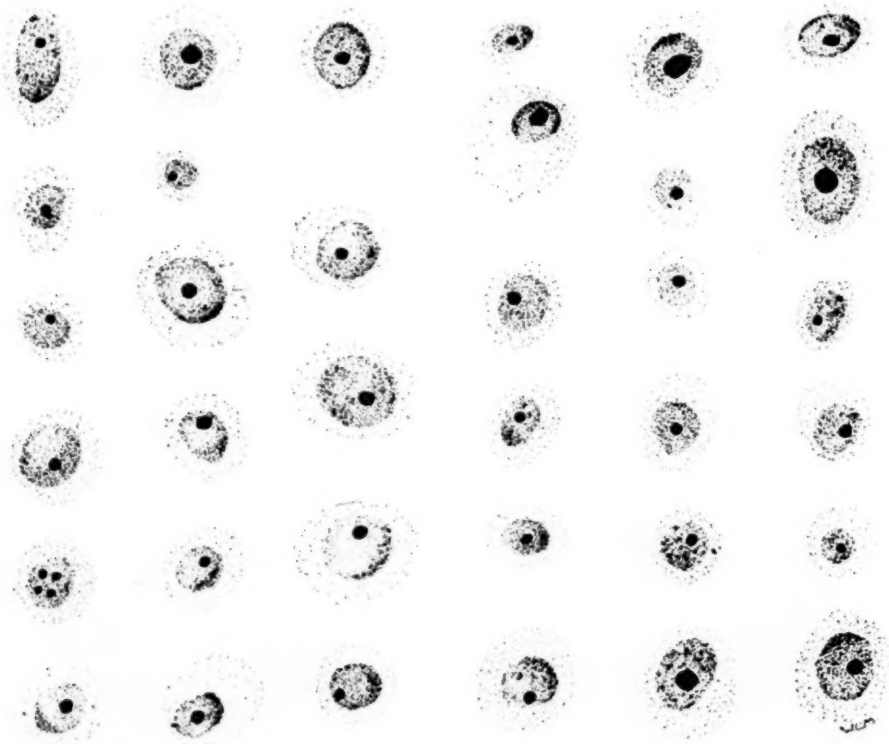

Fig. 6.—Sketches to scale of malignant cells from many sources. They represent those for carcinoma, sarcoma, lymphosarcoma, lymphatic leucemia, and malignant endothelioma. It is impossible to differentiate one from the other, but each one has a morphology unlike any normal regenerative or adult cell in the human body.

of undifferentiated cells. The last picture is the one we call cancer; the cells are of the type seen in Figs. 5 and 6.

The question arises: Has the malignant cell a morphology by which it may be recognized? Textbooks describe the cancer cell as having an irregular shape with an irregularly shaped nucleus which takes the stains densely and frequently shows an asymmetrical mitotic figure. They rarely, if ever, say anything about the nucleolus. This description applies to those cells in pathologic tissues which have been dead for some time, have undergone cytologic changes coincident to and following death, and have been fixed in strong solutions and then embedded in celloidin or paraffin. It is not the

picture which one sees in living tissues or tissues which, although dead, have just died and are studied in an unfixed condition with oil lenses. *Under these less destructive conditions the cancer cell is an ovoidal or spheroidal body with no irregularities of "cell wall," nucleus or nucleolus; the demarcations of the component parts of the cell are perfectly sharp and distinct; the granules of the cytoplasm and nucleoplasm are fairly uniform in size; the mitotic figures are sometimes multipolar and they are never asymmetrical and irregular in my experience.* The whole cell when studied in the fresh condition is the object of study; it is not cut in planes. Its constituents are not coagulated and are, therefore, transparent or translucent; there is no necessity for thin sections such as one attempts to obtain with celloidin and paraffin methods.

The cancer cell may perhaps not always be distinguished from a normal regenerating cell, but this can be done frequently because there is a difference in volume-relationship between nucleolus, nucleus, and the whole

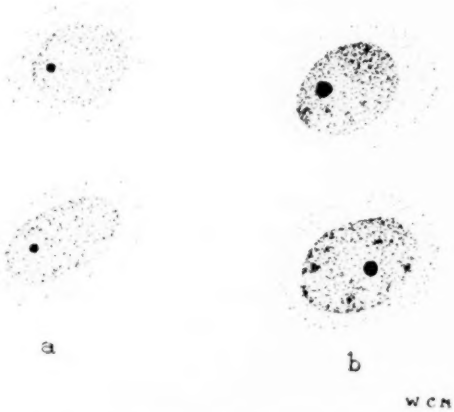

Fig. 7.—Diagrammatic sketches of reparative regenerative cells and malignant regenerative cells to show some of the differences in morphology. The reparative cells are more delicate, have smaller granules, and smaller nucleoli.

cell in the reparative regenerative cells and malignant regenerative cells. The nucleolar-nuclear area ratio is from approximately 1 to 23 in malignant cells and 1 to 50 or more in reparative regenerative cells. There is also a difference in the density of the nucleoplasm and cytoplasm in the extreme exemplars of these two cells. The regenerative cell is more delicately constructed (Fig. 7), and its nuclear granules are usually finer; hence, the nuclear outlines are more delicate. These are some of the differential points. There are qualities which words cannot describe. One learns to recognize the types of cells from experience with actual clinical proof of diagnosis and prognosis just as one recognizes members of his family and friends. It is not always possible to describe our friends in such a manner that others can recognize them. Expertness in the differential recognition of malignant cells, reparative regenerative cells, and adult cells comes through constant contact with them, checked by clinical proof of diagnosis. The problem is one of cytology, not histology. The malignant cells which have just been described are

malignant because they invade the surrounding tissues, spread to distant parts of the body where they multiply independently, disturb the vitality of the whole organism directly and indirectly, and eventually cause death.

One of the greatest problems of the clinicians and pathologists is prognosis, and data upon which it may be made have been largely compiled from statistics based on the average length of life from the time the physician or surgeon sees the patient. At best it is based upon impressions and some personal opinions since there are so many variables and unknown factors in the problem. In general, one considers the following factors which seemingly, if not definitely, influence the relation of newgrowth to longevity: size of growth, cellular character of growth, age of the host, duration of the lesion, relation of growth to nutrition, emaciation in relation to food intake, proximity of growth to vital structures, lymph-glandular involvement (regional and distant), distant organic metastases, multiplicity of the lesions, character of previous treatment, and the morale of the patient.

The influence of these factors is purely relative and very general, and we find, in practice, failures when either good or bad prognoses are given. Recognition of the possibility of prognostic incorrectness has led many wise physicians to give guarded prognoses; or they merely attempt, in diplomatic manner, to improve the morale of the patient and very frankly warn the relatives. This humanitarian and necessary habit of our profession, great and noble as it is, should not prevent us from a strenuous search for facts which lend efficiency to practice since, in this day of economic expertness, the human individual wants to know, frequently, the time left for the completion of his plans of life.

The facts, presented here, are the result of unsatisfactory experience with the lack of definiteness in prognosis. Thus, patients with large cancers of the stomach (or some other organ) with complete regional lymph-glandular involvement have been given the worst prognosis routinely, and those with small lesions and no glandular involvement a better prognosis. While this method of consideration has been good in general, there have been many instances of unexpected and quite surprising variations from the general rule; some patients with small lesions and no apparent glandular involvement die of recurrence very rapidly; others, with large and extensive lesions, live long—sometimes ten and fifteen years—after local removal.

Besides the factors which have been mentioned as being possible aids in formulating prognosis, there are others which can be and have been studied. They are, however, of value only after removal of the neoplasm since they are microscopic in character. The four factors which have thus far been studied are *lymphocytic infiltration*, *fibrosis*, *hyalinization*, and *cellular differentiation*, the last factor being merely an index of the favorableness under which the invading cells are living. By lymphocytic infiltration is meant that infiltration which is in immediate contact with the cancer cells. This intimate contact of reaction is also essential in recording the relation of the reactions of fibrosis and hyalinization. These three reactions to the presence of malignant cells occur in sequence; lymphocytic infiltration is first, fibrosis, second, and hyalinization, third. By cellular differentiation is meant the structural

cellular modification coincident with special function. There are degrees of this differentiation recognizable in the normal evolution of specific tissues, and these stages are frequently seen in the study of neoplastic cells (Fig. 3). Thus, the cells of a so-called adenocarcinoma or a squamous-celled epithelioma are in one of two stages of partial differentiation. Biologists have recognized that the power of growth is indirectly proportional to the height of the differentiation, and this law holds good very apparently for the cells of neoplasms. These four factors are visible, and in the work presented here, have been studied and recorded without any knowledge of the actual length of life in each case. After the observations in the neoplasms had been recorded, they were assembled with the facts pertaining to postoperative life.

There are several facts of scientific interest and practical importance visible in these observations:

Whenever the factors are present, the average length of life is greater than when they are absent. This is true in all four groups of cases studied.

When all of the factors are present, the average length of life is much greater than when all of the factors are absent.

The variations of frequency of the presence of the different factors in different organs is of interest and suggests that the defensive mechanism, while a general phenomenon, acts differently in different regions of the organism.

To this series of observations may be added the results found by Broders in the study of epithelioma. Based upon the degrees of cellular differentiation described by the writer, he has very successfully attempted prognosis and divided the epitheliomas into four clinical groups, Group I being the most favorable and Group IV being the least favorable. Despite the fact that his clinical group numbers are in reverse order of the actual stages of differentiation in relation to rapidity of growth, the facts of growth relationship are according to the biologic law. His numbers I, II, III, and IV represent clinical groups and not the order of degrees of differentiation (0°, 1°, 2°, 3°).

That these are all of the defensive factors in the organism is not to be expected, but that they are all factors which form a part of any key to prognosis can hardly be questioned from the uniformity of the results of observation.

It may be correctly said that the Master Key to diagnosis of malignant and benign neoplastic conditions and inflammatory conditions and prognosis is a checked experience with the differential detailed morphologic characteristics of adult tissue cells, reparative regenerative cells, and neoplastic cells. All of these may be recognized in the fresh unfixed condition with or without stain if the oil lens of the microscope be used. Freezing methods and the different staining methods are only means of decreasing time of search. They, in themselves, will not make great and reliable diagnosticians or prognosticians any more than a good box of colors, a piece of canvas, some good brushes, and an easel will make an artist. I mention this because more emphasis has been laid by writers and talkers on the method than on the necessary training and experience. For the benefit of those who are

always hunting short cuts, I must say, after long and constant experience and study, that there is no short cut to becoming a great clinical pathologist. The subject cannot be learned from books any more than the art of literature or painting can be learned from books. It is the short-cut pathologists who have thrown discredit upon tissue pathology which in truth is, after all, the basis of our knowledge of all disease. May I say also that no one individual can possibly be efficient in tissue pathology, bacteriology, serology, immunology, radiology, roentgenology, chemical pathology, parasitology, and general clinical microscopy. And still this is what is expected by the great majority of hospitals. They even expect it from young men and women. It cannot be done, and it should be discouraged by all members of our profession. The differentiation of pathologic conditions and their clinical interpre-

	STOMACH 99 CASES years	BREAST 92 CASES years	RECTUM 102 CASES years	SKIN 29 CASES days
Average length of postoperative life with differentiation	2.73	3.65	1.54	534.1
Average length of postoperative life without differentiation	2.56	2.37	1.08	274.7
Average length of postoperative life with lymphocytic infiltration	2.73	2.51	1.57	496.2
Average length of postoperative life without lymphocytic infiltration	2.7	2.48	1.31	346.6
Average length of postoperative life with fibrosis		2.72	1.53	655.7
Average length of postoperative life without fibrosis		1.87	1.29	295.6
Average length of postoperative life with hyalinization		2.81	2.33	449.6
Average length of postoperative life without hyalinization		2.21	1.44	437.9
Average length of postoperative life with differentiation and lymphocytic infiltration	2.8	3.78	1.59	644.5
Average length of postoperative life without differentiation and lymphocytic infiltration	1.55	2.45	0.71	204.0
Average length of postoperative life with differentiation and fibrosis		3.87	1.58	808.3
Average length of postoperative life without differentiation and fibrosis		1.96	1.15	257.5
Average length of postoperative life with differentiation and hyalinization		4.0	2.33	587.0
Average length of postoperative life without differentiation and hyalinization		2.04	0.61	257.5
Average length of postoperative life with lymphocytic infiltration and fibrosis		2.69	1.65	739.8
Average length of postoperative life without lymphocytic infiltration and fibrosis		1.4	1.17	155.2
Average length of postoperative life with lymphocytic infiltration and hyalinization		2.76	2.25	404.0
Average length of postoperative life without lymphocytic infiltration and hyalinization		1.68	1.27	255.5
Average length of postoperative life with fibrosis and hyalinization		2.89	2.33	453.8
Average length of postoperative life without fibrosis and hyalinization		2.05	1.28	282.5
Average length of postoperative life with lymphocytic infiltration, differentiation, fibrosis, and hyalinization		4.4	2.25	444.6
Average length of postoperative life without lymphocytic infiltration, differentiation, fibrosis, and hyalinization		1.52	0.76	54.0

tation in the light of the best interests of the patient is an art. Like any art it involves knowledge of technical details, experience, and a broad conception of disease.

DISCUSSION

Dr. B. T. Terry.—It has been my pleasure to work in Dr. MacCarty's laboratories since June 14, 1926, and many times I have seen him make diagnoses of malignancy either from one cell or from a very small number of cells. To do this, however, very great experience is necessary. In spite of the fact that I have tried conscientiously for nearly a year to learn the criteria Dr. MacCarty uses, I am not yet sure of malignancy from the study of one cell only. An example may be worth while. Not long ago a freshly removed lymph node was brought for diagnosis. In the first frozen section one cell was found which Dr. MacCarty regarded as malignant and at once reported it as such to the operating room. Dr. MacCarty pointed out the cell to me. I studied it carefully and thought I saw what he did, but, lacking his experience, I could not be sure of malignancy from this cell alone. Other sections of the same node were then cut and studied and in some of these there were large enough groups of cells for me also to be sure. As the cytologic diagnosis of malignancy now requires an enormous amount of experience, I am hoping that Dr. MacCarty will continue his work, and that he will be able so to simplify his criteria that he can save us the labor of learning forty-five different cells. If he can teach us a simple, sure, and easy way of recognizing malignancy by the study of a single cell, the entire world should be grateful to him.

DIFFERENTIAL BLOOD COUNTS

A Comparison of the Accuracy Obtained by Various Methods*†

By Dean N. Beacom, M.D., Denver, Colorado

A DIFFERENTIAL blood count is one of the most common and also one of the most important of routine laboratory procedures. The question has no doubt arisen in the minds of many as to the accuracy of these counts since authors of various texts on clinical laboratory methods do not agree as to which method of making a blood film gives the most satisfactory spread for such work. In his recent book Haden[1] says, "Blood films may be made either on cover glasses or on slides. For careful work, preparations on cover glasses are far preferable. For the accurate differential counting of the leucocytes such preparations are essential." Cummer[2] prefers the use of cover-slips over slides because in his opinion the leucocytes are unevenly distributed in the latter case. Other writers, Kilduffe[3] and Simon,[4] prefer the use of slides over cover glasses for the preparation of blood films.

During the summer of 1926 a questionnaire was prepared and sent to various laboratory directors. Among the questions asked were those pertaining to the method used in making the blood film, the part or parts of the film counted, and the number of cells routinely classified for a differential blood count. While only eighty-two answers were received, they probably give a fairly good idea as to the methods used in this country. Seventy-five workers use slides in the preparation of blood films, while only seven use cover-slips. Of this majority about 60 per cent place the drop behind the spreader slide, while about 30 per cent have the drop ahead and either push or drag the cells across. The remaining 10 per cent is composed of those who use the cigarette paper method or who have special methods which in general may be considered as a combination of the first two mentioned.

It was learned that fifteen of the laboratories replying classify only one hundred cells for a differential count. Twenty-three observed two hundred cells; three, three hundred; and three, five hundred; while the remainder, which comprises about 50 per cent, classify from one hundred to one thousand cells, depending upon the accuracy desired. A majority of this latter group, however, count not more than three hundred leucocytes.

Since there is a difference of opinion as to the best method of preparing a blood film for differential counting, and also a difference as to the number of cells which should be classified, an attempt was made to see, (1) if one method was more accurate than another, and (2) to learn how many cells should be observed for an accurate differential count. In order to arrive at

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the conclusions given, more than two hundred thousand leucocytes were studied.

In general the first question was attacked in the following manner: Two preparations were made, one by a method known to be accurate when every leucocyte was counted, and the other by one of the methods which was to be tested.

Since none of the slide methods were known to be absolutely accurate, due to the fact that certain cells might adhere to the spreader slide, the cover-slip method was tried. But here it was learned that films made in this manner were usually unsatisfactory either because of ruptured cells or because of areas in which the erythrocytes were so thick that the leucocytes could not be classified.

Fairly satisfactory films were usually obtained by using two slides in a manner similar to the two-cover method, but too often the preparations were not good, owing to differences in curvature of the glass.

An accurate method was then developed which was found to be very satisfactory and which may be called "The Slide and Cover Method." The technic is very simple, and it is practically impossible to obtain a film in which all of the cells cannot be counted, provided that certain simple rules of technic are followed. A small drop of blood is placed near the end of a clean slide, and a medium to thin cover-slip is dropped on the blood, which will then flow out in a thin layer if the glass is clean and if there is not too much curvature to the slide. If the film does not flow out properly, very gentle pressure will bring this about. As soon as the blood has stopped flowing, the slide is held on the table with the left hand, and the first two fingers of the right are placed near the edges close to the left end of the cover glass. Then with an even, fairly rapid pull it is slid along the slide in the direction of its long axis. The amount of pressure necessary is only enough to keep the fingers from slipping from the spreader. This leaves a smooth, even film covering a large or small area of the slide, depending upon the size of the drop used. It is interesting to note at this point that only very rarely are any cells found on the cover-slip, and when this does occur, the differential percentage of leucocytes is the same as that found on the slide. The use of two fingers in sliding off the cover is very necessary for two reasons. If a convex slide is being used, a finger on each side of the glass will cause it to conform better with the curvature of the slide and thus allow a more uniform film. If only one finger is used, there is a tendency for it to slip off. When there is a second attempt at removal, it will be found that the adhesion is so great that many of the polymorphonuclear leucocytes will be ruptured, a condition very commonly observed in cover-slip preparations.

After developing a method known to be accurate, certain observations were made in order to be sure that the results had been properly controlled.

It was found by trial that it was not necessary to observe every leucocyte on a slide in order to obtain an accurate differential count. Several slides were prepared by the slide and cover method, every cell counted, and the true percentage of polymorphonuclear leucocytes was found. Then each slide was recounted, classifying the cells in every other row across each film, the

edges of which were always included. In every case it was found that the neutrophilic percentage checked within 2 per cent of that found in the first instance. Consequently, all of the counts recorded in this paper represent the leucocytes found in every second row across each film.

A skin puncture was made and four films prepared at minute intervals from each of two normal individuals. A great deal of milking of the finger was necessary in each case in order to obtain enough blood for the fourth smear. When every second row of each preparation was counted, the differences between the highest and lowest polymorphonuclear findings were less than 1.5 per cent.

Although we know that whenever the skin is injured there is a gathering of certain leucocytes at that point of injury, this occurrence either is not rapid enough or not marked enough to make any difference in the blood picture as it is obtained routinely. Also since in both cases squeezing the finger was necessary in order to obtain blood for the fourth film, that fact makes no difference in the results of a differential blood count, although we know that it does cause an error in total counts.

Neither does the size of the drop used have any effect. Small films, having less than two thousand leucocytes, were made, and then some were made having more than seven thousand. The results obtained in these cases always corresponded within 2 per cent.

Having answered some necessary questions, the accuracy of the more commonly used methods of preparing blood films was checked in the following manner: The finger was punctured in the usual way, the first drop of blood wiped off, and a smear was made on a slide using the above known accurate slide and cover method. Then one or more films were prepared from the same puncture by the method which was being tested for accuracy.

The four methods tested in this manner were: (1) *The drop behind the spreader method.* This, in brief, consists of placing the drop of blood behind

TABLE I

ACCURACY OF RESULTS WHEN BLOOD IS PLACED BEHIND SPREADER. POLYMORPHONUCLEAR PERCENTAGE

(every second row across each film counted)

PATIENT	SLIDE AND COVER METHOD	DROP BEHIND SPREADER ALL BLOOD USED	REMARKS
M. O.	60.5	61.3	Many cells at end
F. D.	55.0	54.5	Many cells at end
W. B.	66.3	65.1	Many cells at end
		ALL BLOOD NOT USED	
F. D.	54.4	53.1	Thick film
M. P.	62.3	62.6	Thick film
F. D.	51.7	50.1	Thick film
L. K.	66.8	60.2	Medium film
M. O.	63.6	61.9	Medium film
F. D.	51.9	43.5	Thin film
L. P.	67.5	51.4	Thin film
E. M.	55.4	49.0	Thin film
H. H.	58.1	49.8	Thin film
L. P.	63.8	58.4	Thin film

the spreader slide which is held at an angle of about 40°. This is then pushed forward, and the blood follows leaving a smooth, even preparation. (2) *The dragging method*, somewhat similar to the first, except for the fact that the drop is placed in the acute angle in front of the spreader slide and the cells dragged across. (3) *The pushing method*, having the drop of blood placed in the obtuse angle in front of the spreader which is then pushed forward across the slide. (4) *The cigarette paper method.* These films are prepared in the manner described for the dragging method, except for the fact that a cigarette paper is used for a spreader instead of a slide.

The results of the accuracy of these methods are shown in Tables I, II,

TABLE II

ACCURACY OF RESULTS OBTAINED BY DRAGGING METHOD. POLYMORPHONUCLEAR PERCENTAGE
(Every second row across each film counted)

METHOD	PATIENT	REMARKS	P. M. N. %
Slide and Cover	F. D.		52.7
Dragging		All blood used	52.1
		All blood not used	52.9
		All blood not used	55.4
Slide and Cover	H. H.		55.2
Dragging		All blood used	56.5
		All blood not used	54.2
		All blood not used	56.5
Slide and Cover	M. P.		60.3
Dragging		All blood used	61.9
		All blood not used	68.0
		All blood not used	66.3

TABLE III

ACCURACY OF RESULTS OBTAINED BY PUSHING METHOD. POLYMORPHONUCLEAR PERCENTAGE
(Every second row across each film counted)

METHOD	PATIENT	REMARKS	P. M. N. %	
Slide and Cover	F. D.		57.7	
Pushing		All blood used	57.1	
		All blood not used	48.4	
		All blood not used	47.8	
Slide and Cover	F. J.		56.5	
Pushing		All blood used	55.8	
		All blood not used	49.7	
		All blood not used	55.2	(thick film)
		All blood not used	51.8	
		All blood not used	54.1	
		All blood not used	51.3	

TABLE IV

ACCURACY OF RESULTS OBTAINED BY CIGARETTE-PAPER METHOD. POLYMORPHONUCLEAR PERCENTAGE
(Every second row across each film counted)

PATIENT	SLIDE AND COVER METHOD	CIGARETTE-PAPER METHOD
L. P.	61.4	62.7
M. P.	57.9	56.6
F. D.	63.9	64.2
W. B.	66.1	65.4

III, and IV, the figures in which represent the polymorphonuclear percentages obtained when all of the leucocytes in every second row across each film had been classified in groups of one hundred cells.

DISCUSSION OF RESULTS

Table I shows the results obtained when the film was made by placing the drop behind the spreader slide and the findings compared to those of the slide and cover method. It will be noted in the first three counts that when all of the blood was used in making the film, the results checked very closely with the accurate findings, thus showing that certain leucocytes do not adhere to the spreader, and that a differential count made from this kind of smear is accurate provided enough cells are classified. In each of these three slides many leucocytes were found at the extreme end of the film. This fact may be seen in practically every film which is made by means of a spreader slide, no matter what special kind of technic is used. Wright[5] explains this by stating that the plasma, red corpuscles, and the smaller leucocytes are deposited on the slide first, and the larger leucocytes are pushed or dragged ahead of the spreader until all of the fluid has drained away, leaving these cells stranded. This observation will explain very nicely the results obtained in the latter part of the table. It will be seen that in the last ten counts all of the blood was not used in preparing the film. Some of the films were thick, some medium, and some thin; and the thinner the film the lower the percentage of neutrophiles. When a thin film is made, there is a lessened number of small cells deposited, and consequently a large number of large cells are floated to the end; and when all of the blood is not used in preparing the slide, these cells are not found. When a thick film is made, a greater number of small cells and consequently a greater number of large cells adhere, thus giving a more accurate differential count. At this point it is well to explain what is meant by a thick and thin preparation. Those in which the red corpuscles are well separated are considered thin films; those in which the red cells overlap to some extent are called medium ones, and the thick films are those in which the cells are piled up to such an extent that rouleaux formation may be noted.

Table II shows the accuracy of counts made on films prepared by the dragging method. Here again the findings show that cells do not adhere to the spreader slide in such amounts as to interfere with correct results. But contrary to the findings in the first method tested, the percentage of polymorphonuclear leucocytes is not decreased, but usually slightly increased above the correct findings when all of the blood is not used in making the preparation, due possibly to the fact that rather thick films were made in each case.

In Table III it may be observed that when all of the blood is not utilized in preparing the films, the polymorphonuclear percentage is lowered. But even in very thin films this decrease is not as marked as in those prepared with the drop behind the spreader, although it is more pronounced than when the dragging method is used. When films are prepared by this pushing method, some of the blood will be seen to flow under the spreader slide; and

because of this fact the film is really prepared by pushing some of the cells ahead and allowing some to follow. Since this method may be considered as a combination of the two preceding ones discussed, the results are those which might be expected when all of the blood is not utilized in preparing the slide.

The cigarette-paper method is an accurate one as shown in Table IV. Its chief disadvantage is that many neutrophiles are found at the edges and at

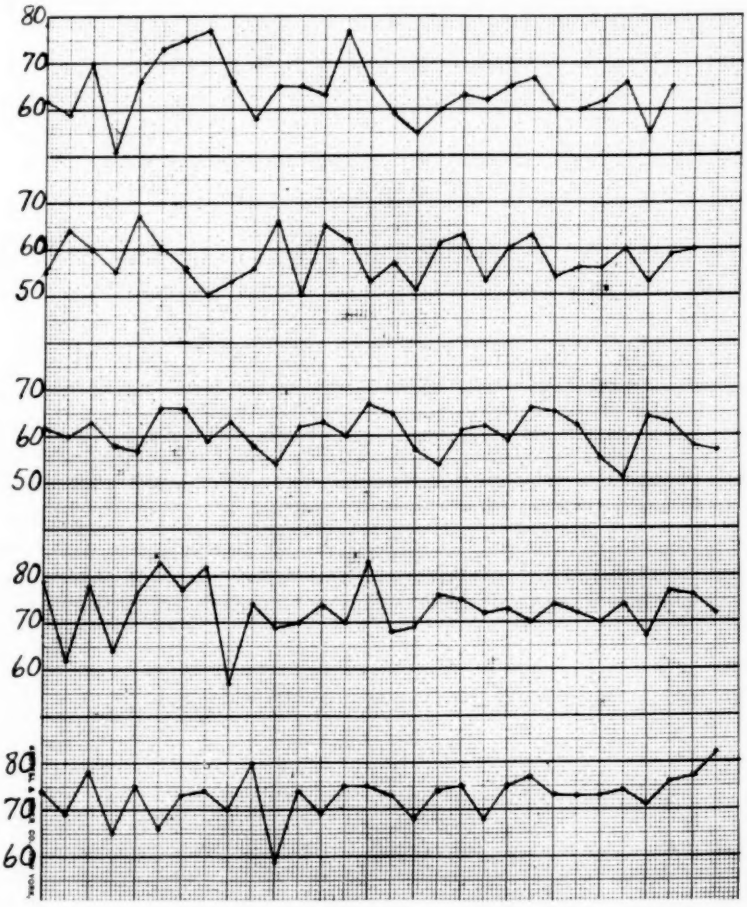

Chart 1.—Percentage graphs of neutrophilic content of five cover-slip preparations in consecutive counts of 100 leucocytes. (Every second row across each film counted.)

the end of the film because they have been floated out as the cigarette paper has been pulled across the slide.

Films made by the cover-slip method were then tested for accuracy, and the results showed that preparations of this kind were satisfactory for differential counting. However, I cannot agree with those who say the leucocytes are more evenly distributed on cover-slips than on slides. A large number of cover-slip films were counted in groups of one hundred cells, and the results brought out the point that there was as great a variation in the neutrophilic percentage as there was in slides prepared by any of the methods

in common use. If one counts every leucocyte on both covers, he will always find a variation of at least 15 per cent between the highest and lowest polymorphonuclear percentage. He will not find this difference to be any greater in films prepared on slides if the proper kind of technic has been employed.

Much better technic is required to make satisfactory cover-slip smears than those made on slides. When I began this work, I was unable to obtain constantly what I considered to be a good cover-slip preparation. I thought that the variation in percentage obtained might be due to the fact that my films were not as smooth as they should be. I then wrote letters to the various workers who had said in the questionnaire that they were using cover-slip preparations for differential counting, telling them of this difficulty, and asked them to send what they considered to be a satisfactory film. Several specimens were obtained which were counted, and the variation was found to be as great as in the previously counted ones. The results of a few representative counts are shown in graphic form in Chart I.

Because of the time required to count every cell or even half of the cells on a slide or cover, it is not practical to use such a method in routine work. The true percentage of cells was obtained from each slide when the first part of the work had been done, and an attempt was made to learn whether or not a fairly accurate percentage could be obtained by counting three groups of one hundred cells each on different parts of the film. Two of these counts were made on each film, and the results were compared with those obtained when every second row had been examined. The first count was made in the following manner. One hundred leucocytes were classified in each of three areas extending across the film, one near the beginning, one at the middle, and one near the end of the preparation, and the average of the three counts obtained. The other count was made by examining three areas along the long

TABLE V

ACCURACY OF RESULTS WHEN 300 CELLS ARE CLASSIFIED. POLYMORPHONUCLEAR PERCENTAGE

METHOD USED	PER CENT WHEN EVERY SECOND ROW HAD BEEN COUNTED	PER CENT WHEN 300 CELLS HAD BEEN COUNTED THROUGH SHORT AXIS OF FILM	PER CENT WHEN 300 CELLS HAD BEEN COUNTED THROUGH LONG AXIS OF FILM
Slide and Cover	58.4	61.0	63.3
Slide and Cover	61.3	60.7	57.0
Slide and Cover	60.8	61.0	57.3
Slide and Cover	54.4	54.3	55.7
Slide and Cover	53.1	55.0	55.7
Slide and Cover	51.7	50.3	48.3
Slide and Cover	51.4	62.3	62.0
Slide and Cover	57.9	55.3	55.7
Slide and Cover	63.9	62.0	65.0
Slide and Cover	60.3	58.0	58.7
Slide and Cover	57.7	56.3	59.3
Drop behind spreader	50.1	55.7	49.3
Drop behind spreader	60.2	60.3	57.0
Cigarette-paper	65.7	58.7	65.3
Cigarette-paper	56.6	53.7	57.3
Cigarette-paper	64.2	58.0	64.7
Dragging	68.0	65.0	66.0
Dragging	61.9	61.0	59.0
Dragging	66.3	64.0	63.0
Pushing	55.2	52.3	55.0

TABLE VI

SHOWING HIGHEST AND LOWEST P. M. N. PERCENTAGE IN EACH OF FIVE FILMS MADE BY VARIOUS METHODS WHEN EVERY SECOND ROW HAD BEEN COUNTED

METHOD	HIGH	LOW	DIFF.	HIGH	LOW	DIFF.	HIGH	LOW	DIFF.	HIGH	LOW	DIFF.	HIGH	LOW	DIFF.	AVE.
Slide and Cover	67	51	16	65	54	11	68	54	14	64	53	11	57	67	10	12.4
Drop behind spreader	73	52	21	75	51	24	73	53	20	72	50	22	53	38	15	20.4
Dragging	78	61	17	69	52	17	73	57	16	64	50	14	73	46	27	18.2
Pushing	61	47	14	58	42	16	71	46	25	64	41	23	68	50	18	19.2
Cigarette-paper	74	60	14	78	48	30	70	43	27	70	60	10	71	61	10	18.2
Cover-slip	77	51	26	67	50	17	67	51	16	83	57	26	82	59	23	21.6

axis of the slide. The results of some of these counts may be seen in Table V which represents differential counts from various methods of preparing the film.

It will be noted that an exact count is seldom obtained when only three hundred leucocytes are studied, but since most of the counts do not differ more than 3 per cent, it makes little difference from a clinical standpoint. This slight error will be found only if good technic has been used in preparing the film and the proper areas counted. It is maintained by some that great numbers of cells are found at the edges of a smear which has been prepared by a two slide method. But a condition such as this is seen only when the technic has not been good. When working with any two-slide method, if the film is begun before the drop has completely flowed along the spreader slide, many polymorphonuclear leucocytes will be found along the edges. The same thing will occur if too large a drop has been used. It is true that large numbers of cells are found near the end of a film prepared on a slide by the usual methods when all of the blood has been employed in the preparation. This will cause an error in differential counting unless one of the three groups classified has been obtained from this area. If this third part of the film has not been considered, the neutrophilic percentage will be somewhat lower than it should be.

Because of the fact that many blood films are not prepared or counted in the proper manner, it is undoubtedly true that the slide and cover method holds several advantages over others. The technic is simple. Ruptured cells in excess may be found, but this is due to the fact that either too great pressure has been placed on the cover or it has not been removed with an even, fairly rapid movement. Even when ruptured cells are found, the number is not as great as that observed in many cover-slip preparations. The leucocytes are more evenly distributed than in other methods as may be seen in Table VI. Large numbers of cells are not found either at the edges or at the end of the film. When every cell on a slide is classified, one will not see as great variation in percentages as is usually observed in the more commonly used methods. But even though the cells are more evenly distributed, one cannot be sure that he is accurate within 10 per cent when only one hundred cells have been observed. The method utilizes all of the cells in the drop of blood, and so one can be sure that none of the large leucocytes have been floated out to the end and allowed to dry in an area too thick to be counted.

CONCLUSIONS

Blood films properly prepared on slides give an accurate differential count provided enough leucocytes have been classified and all of the blood has been utilized in making the preparation.

If all of the blood in the drop has not been used in making the film, the neutrophilic percentage will be decreased proportionately to the thinness of the smear.

A differential blood count on a film prepared by any of the methods previously discussed is seldom accurate within 10 per cent if only one hundred cells are classified.

A differential count made from either slides or cover-slips is accurate within 3 per cent if correct technic has been employed in preparing the film and if three hundred cells in three different areas have been studied.

Leucocytes in cover-slip preparations are not more evenly distributed than in those made on slides.

A method for the preparation of blood films is presented which is simple, accurate, and gives a more even distribution of leucocytes than is obtained by other methods.

I wish to thank Dr. James C. Todd and Dr. E. R. Mugrage for valuable suggestions and criticisms during the progress of this work, and also Mrs. Frances Dunning for technical assistance.

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DISCUSSION

Dr. A. H. Sanford.—I was told of this method by Dr. Todd a few months ago and was pleased to find out how simple it was. I believe that those of you who are teachers of clinical pathology will find it worth while to demonstrate this method to your students and observe the results of its use in their hands.

Dr. Robert A. Keilty.—I would like to ask one question. Is this to be an actual routine for one who has to count 15, 20, or 30 differential counts in a day, using three hundred fields to each slide? I would like to ask the reaction to this question.

Dr. F. B. Johnson.—It is a problem to decide the most accurate way to do these counts. I realize more than you would otherwise this error in dealing with my students. This is a new method, and I will be glad to try it out. I have been trying out a method in making differential counts, using the same dilution that we make our total counts from. The disadvantages are that it is very hard to differentiate an eosinophile from a polymorphonuclear leucocyte. However, I think if the method, introduced here by Dr. Beacom, is followed out and checked by this method we could get a fair idea of the accuracy. Undoubtedly when we use the dilution of the blood for the total counts, we get a more even distribution of the cells, and therefore the differential counting is more accurate except for differentiating eosinophiles and basophiles from neutrophiles, which in most cases is unimportant.

Dr. Nathan Rosenthal.—I should like to ask if Dr. Beacom noticed any difference between blood smears taken from the finger and from the ear. In certain cases of subacute bacterial endocarditis, macrophages may be found in the ear blood and are seldom present in blood smears taken from the finger. We have found that cover slips are not suitable for the study of abnormal blood cells, and have obtained better results from the use of slides.

Dr. Beacom (closing).—I am very glad that Dr. Sanford has tried out this method. I might state that I brought a few slides and cover slips with me, and I will be glad to show

this technic sometime during the course of the meeting. I did not do any work on blood from the ear. This would involve too much work to bring out the points which I wished brought out. As to the necessity of counting three hundred cells, I don't believe I have anything to say about that, since a count of 100 cells is not enough for accuracy. It seems to me that since we are doing clinical pathology, we should be as accurate as possible. It is true that a count of 55 per cent or 65 per cent polymorphonuclears means nothing whatsoever, but if we have an abnormal case of fifteen or twenty thousand and then get a polymorphonuclear count of 70 per cent or 85 per cent or more, it does mean something. Although we may have a great many slides to count, it is necessary to be as accurate as possible. The slide and cover-glass method I do not give as a remedy for all evils of differential counting. Three hundred cells should be counted. There are too few clinical pathologists, and as a result most of our medical students are going to be located in towns away from laboratories. They are going to have to do their own differential counting. The cover-slip method is very difficult to learn, and a medical student will not learn it. I believe a simpler method is worth while.

PATHOLOGIC LABORATORY EXAMINATIONS FOR THE DENTIST*

By Charles G. Darlington, M.D., New York City

WHILE the title of this paper is given as Pathologic Examinations for the Dentist, it would have been better but too lengthy to have titled it "A Plea for Instruction of the Dentist in the Value of Pathologic Examinations."

With that object in mind, it is not my intention to burden you with a list of clinical laboratory procedures with their indications in dentistry, but rather to call to your attention an imperative duty that rests with us to teach the dentist correctly what he may expect from the use of many of our laboratory procedures.

At first thought the teaching of pathology to the dentist seems an unusually large undertaking, but not a hopeless one, in fact a rather encouraging one; but first let us consider the size of our problem.

Some comprehension of the size of the problem can be gathered from a realization of the fact that the average dentist today knows little pathology and least of all does he know of the various pathologic examinations that might be of inestimable value to him. Before we are too hasty to condemn him for his shortcomings along this line, however, we must realize that we can only condemn him according to his opportunities; therefore, on that account he deserves sympathy rather than censure as he has had little instruction in pathology, at least as compared with his medical confreré.

Now realizing that our undertaking is large, let us see how the situation is not hopeless but rather encouraging.

First, I find it encouraging, because in my experience in teaching pathology to graduate and nongraduate students the past two years at New York University College of Dentistry, I have found particularly the graduate student most anxious for knowledge along these lines. Although we must admit

*Read before the Sixth Annual Convention of the American Society of Clinical Pathologists in Washington, D. C., May 13, 14, and 16, 1927.

that while unfortunately we have not found all dental students eager for pathologic knowledge, each year we can see distinct improvement.

Next, even more encouraging is the recent trend of dental education. Not many years ago the dental student was taught little or no pathology as almost all his teachings were exerted toward the mechanical side of his work. However, of recent years especially, there has been a revolutionary tendency toward emphasizing that the dentist was not just a mechanic, but, as he was working on the human body and as it was recognized that many of his patients' ills might be primary or secondary in the mouth, the doctor of dental surgery must needs have a pretty thorough understanding of much of medicine.

This has directly modified the teaching of dentistry, and naturally on this account the teaching of pathology has come more and more to the fore, and the curricula of the various dental schools are taking this into consideration. While the necessity for more extensive instruction in pathology and the other fundamental medical sciences is somewhat resented by many of the older dentists because the dentists of the past have got along perfectly well without such instruction, competition is keen, and the younger men and the older progressive men are really not only eager but anxious for such instruction.

General pathology is being taught in the dental schools, but I do not believe the teaching of clinical pathology or the value of various pathologic examinations is being taught proportionately; hence, this paper is written.

I do not desire to make clinical pathologists of the dentists, but a proper understanding of how and when to make smears and cultures, what assistance various laboratory examinations may render in establishing diagnoses, in particular the value of blood counts, Wassermanns, biopsies, bleeding and coagulation tests, urine examinations, etc., is a definite thing that we should make it our business to have taught.

As remarked previously in this article, it is not my intention to burden you with a list of clinical laboratory procedures with their indications in dentistry, but in order to help explain my previous remarks, I shall select and briefly comment on urine examinations, coagulation and bleeding time tests, examinations of value in syphilis, and the biopsy.

Preliminary to this, however, illustrative of the necessity for such instruction, I might put under the caption of foolish questions, remarks, or requests (remarks or requests we frequently meet):

Take a blood count for syphilis, meaning a Wassermann.

Take a blood count for syphilis, meaning demonstration of Treponema pallidum.

Take a blood smear for syphilis, meaning a Wassermann.

Take a blood smear for syphilis, meaning demonstration of Treponema pallidum.

A similar request from a smear of an oral lesion.

Smear for the diagnosis of anything.

Culture for the diagnosis of anything.

"That patient cannot have syphilis because the Wassermann is negative."

URINE EXAMINATIONS

As I do not wish to omit the value of the simplest of laboratory examinations, urine examinations, I shall mention them, but it is not my intention to discuss them at this time. Sufficient for the present, is the emphasis that a general knowledge of the interpretation of special abnormal elements, as pus, blood, bacteria, casts, albumin, and sugar, the last mentioned especially, may show the intelligent dentist much.

COAGULATION AND BLEEDING TESTS

I have purposely included the value of coagulation and bleeding time tests to the dentists, because I am not quite sure just how valuable they are, or better, how valuable they might be.

About a year ago Dr. Leo Winter and I published an article,* in which we called attention to this procedure in oral work, and reviewed two hundred coagulation and bleeding tests, many of which were made both before and after suprarenin and novocaine injections. The method used was Goeckel's, a method somewhat similar to Duke's method, which has the decided objection as well as advantage of being a skin puncture method. We at least showed that the injection slightly shortened the coagulation time. In that series there were no postoperative hemorrhages, but since that time we have had two interesting cases.

CASE 1.—12/15/25. C. T., aged seventeen, had tooth extracted six days previously. Five days after extraction during the night bleeding started and continued until morning. Patient came to the clinic, and tests were performed with these results: bleeding time—three minutes, thirty seconds; coagulation time—ten minutes.

CASE 2.—12/15/25. F., aged twenty-four, gave a history of extraction of teeth about two weeks previously, following which she bled for three days. Tests were performed two weeks after extraction with the following results: bleeding time—three minutes, ten seconds; coagulation time—nine minutes, forty seconds.

Following the tests, the patient bled from the wound in finger.

Theoretically at least, what we should like to foretell from the test is a tendency toward postoperative hemorrhage. Probably we can tell that a *tendency toward* postoperative hemorrhage exists, but there are so many other extraneous factors that influence postoperative hemorrhage that we know that we cannot tell which patient is going to have a postoperative hemorrhage; however, if not routinely done by dentists, it should at least be done in cases when the patient gives a history of any form of bleeding.

It is the style today in hospitals to do at least coagulation time tests on tonsil and adenoid cases, and I must admit that from hundreds of such tests, mostly performed with this method, but many of them with the capillary tube method (both skin puncture tests, I'll admit) I have yet to notice in these "T and A" cases an abnormal prolongation of coagulation time where postoperative hemorrhage developed, and needless to say, several patients in

*Winter, Leo, and Darlington, Charles G.: Control of Bleeding in Minor Oral Surgery Operations, with Records of Two Hundred Coagulation and Bleeding Tests, Jour. Dental Res., 1924-26, vi, 13.

whom postoperative hemorrhage developed showed normal coagulation times; however, the latter are more easily explained.

Perhaps had venipuncture methods been used, our results would have been better, but obviously for such a type of case, or in routine oral work, venipuncture methods are not practicable.

EXAMINATIONS OF VALUE IN SYPHILIS

Not only are the fundamentally acquired and congenital syphilitic lesions much misunderstood, but more so are the laboratory procedures of aid in their diagnoses misunderstood; misunderstood as to how they are to be performed (material used, etc.) and misunderstood as to interpretation. In this respect let us consider dark-field illumination, the Wassermann, and the biopsy.

Dark-Field Illumination.—The fact that this examination is for the presence of the causative agent of syphilis, the Treponema pallidum, is not well enough understood, and in the same way the knowledge of which lesions, such as chancre, mucous patches, and gummas (active syphilitic lesions), are likely to harbor the organism and, therefore, should be subjected to such an examination, as well as the necessity for wet material and the significance of a negative examination, are also not sufficiently well realized.

Wassermann.—The dentist's knowledge of the Wassermann test is largely confined to the blood Wassermann, and needless to say, his impression that a negative test means no syphilis while a positive test (including one- and two-plus reactions) means syphilis, needs correction. Besides this their knowledge of relative agreements or disagreements of the Wassermann with primary, secondary, tertiary, and congenital lues will stand improvement.

Brevity excusing the too arbitrary nature of the statements, let us summarize some general rules for interpretation.

Except in treated cases, only a three- or a four-plus Wassermann is a positive Wassermann.

A positive Wassermann (leprosy and yaws excepted) means syphilis.

Twenty per cent of known syphilities give negative Wassermanns.

Spinal fluid Wassermann tests are often of value in "Nerve Syphilis," where the blood Wassermann tests are negative.

Chancres show only a percentage of positive agreements with the Wassermann test, and these are roughly proportionate to the age of the lesion.

Syphilitic secondary lesions correspond almost exactly with the Wassermann test.

Tertiary syphilitic lesions correspond in 80 per cent of cases with the Wassermann test.

Congenital lesions show negative Wassermanns very frequently, and "inherited" lesions show negative Wassermanns even more frequently.

The biopsy in a given case may be of value in the diagnosis of syphilis alone or in association with nonsyphilitic lesions, which association is not uncommon, or it may be of value in a negative way. I shall speak further of the value of the biopsy.

THE BIOPSY

Last but not least the biopsy needs some consideration. I use the term biopsy here not in its narrow or exact meaning, i.e., the taking of a piece of tissue for microscopic pathologic examination, but to include the microscopic examination of all tissues or tumors (which in many cases may be preferably removed in toto without preliminary biopsy).

From our fairly large experience in examining material from our oral surgery clinic (Dr. Leo Winter, Chief) which represents three hundred and forty tissue examinations, we have been impressed with the following facts. The term epulis (from the gum) as it may include such a variety of conditions (benign giant-celled sarcoma of the epulis type, fibrous epulis, fibroma, inflammatory hyperplasia, angiectasis or angioma, osteosis or osteoma, papilloma, etc.) should not be used. As usually those cases clinically diagnosed epulis are benign giant-celled sarcoma, or a fibrous epulis, or polyp, clinically and pathologically these diagnoses are in agreement, but we have occasionally seen the typical microscopic picture of benign giant-celled sarcoma where clinically the cases were otherwise, as:

1. Clinically an inflammatory reaction but no apparent tumor.
2. Clinically on account of the amount of involvement an extensive malignant looking picture.

The opposite we have also seen, as:

1. Clinically benign giant-celled epulis which microscopically was malignant.
2. Clinically a fibrous epulis which was microscopically malignant.

Carcinomas are not infrequent. Twenty-three such cases have been found out of three hundred and forty cases examined, or an incidence of 6.7 per cent. Twenty of these were squamous, one basal, and one from the antrum.

Of these twenty-three cases, the lip or tongue, "the most common location for carcinomas of the mouth" was affected in only one case—an involvement of the tongue. These twenty-three cases showed involvement of various other parts of the mouth as follows:

Gums	11
Floor of the mouth	5
Antrum	1
Palate	3
Cheek	2

The preponderance of twenty-two males to one female and the relatively higher age level (oldest, eighty-four years—youngest, thirty-one years, an average of fifty-six years) are in keeping with the usual figures for carcinomas of the lip and tongue.

The association with syphilis and the value of the biopsy plus the Wassermann is brought out by Case No. 7406A which clinically gave a history of syphilis and a fairly well circumscribed mass in the cheek with a four-plus Wassermann, leading many to the diagnosis of gumma, and being corrected to squamous-celled carcinoma by performing a biopsy.

Adamantinoma are not as rare as one would expect, ten such cases having been observed.

A knowledge of the clinical picture in conjunction with the microscopic examination is essential in many cases in establishing a diagnosis. This is particularly true of cysts and granulomas. The following case of my chief, Professor Fraser, at the New York University and Bellevue Hospital Medical College, which came to him a few years ago, illustrates this most bitterly. Not knowing anything about the clinical case, a pathologist, upon examination of a microscopic tissue, had made a diagnosis of a round-celled lymphosarcoma, upon which diagnosis, unfortunately, a resection of the jaw was made. With the history and clinical findings, however, it was easily recognized by Dr. Fraser as a simple root lymphogranuloma.

I have selected these last three subjects for special comment only because my oral experience has been chiefly with these problems. The value of the urine examinations, especially for the detection of diabetes, a disease which presents many important dental problems, the too frequent diagnosis of "Vincent's Angina," the value of blood chemical examinations, blood smears, bacteriologic smears and cultures, and the laboratory aids in the diagnosis of focal infections, I shall not comment upon.

CONCLUSION

In conclusion let me add that, naturally, in order to teach the dentist the application of laboratory examinations, those who know them best can teach them best. Does it not seem unfortunately true in this respect that concerning many oral lesions our knowledge is decidedly limited? How few routine laboratory examinations have been made with "routine" oral lesions! Therefore, does it not seem not only possible, but probable that, could at least many of the more simple laboratory examinations be more universally made, much light would be thrown on many dental problems?

75 EAST FIFTY-FIFTH STREET.

DISCUSSION

Dr. Robert A. Keilty.—I do not like to be a chronic discusser. This is a most important problem; one which we have very much neglected as was brought out this morning in the case of urine. We cannot go into detail too much. We have neglected this, and the dentist ought to know these things. The internists are demanding some work on it, and it is up to the laboratory man to do a great deal of this work on this matter. When anything concerns pathology or bacteriology, it rightly belongs to the laboratory man.

Dr. Wm. G. Exton.—I would like to ask Dr. Darlington how far he thinks the clinical pathologist should go, working with the dentist without having a physician in the case. Some dentists go on treating doubtful conditions in the mouth by using all sorts of antiseptics; they diagnose general conditions, sending urine to the laboratory, etc., and act as if they were the proper persons to treat doubtful cases. I would really like to know just how far Dr. Darlington thinks the dentist should go and how far he thinks the clinical pathologist should go in working alone with the dentist.

Dr. Darlington (closing).—As for the dentist going ahead treating cases without an attending physician, there is not a tendency to do this. They are only too glad to get the advice of the clinical pathologist. We should give them this advice.

TURBIDIMETRIC METHODS FOR SUGAR IN BLOOD AND URINE[1*]

By Anton R. Rose, M.S., Ph.D., Newark, New Jersey

IN ALKALINE solutions, potassium ferricyanide is reduced to ferrocyanide by glucose, and this latter can be quantitatively separated from the excess ferricyanide by precipitation with zinc or silver salts in dilute ammoniacal solutions. Under optimum conditions, the fine suspension thus formed is quantitatively reproducible and very suitable for turbidimetric measurement.

The analysis is carried out as follows: (A) clarify by shaking sample with a modified Lloyd's reagent;[2] (B) add 3 c.c. of ferricyanide reagent[3] to 1 c.c. of the filtrate; (C) digest for fifteen minutes in boiling water;[4] (D) dilute with water to the 20 c.c. mark and mix; (E) to 1 c.c. of this diluted mixture add 13 c.c. of 4 per cent ammonia solution and treat with 1 c.c. molar zinc sulphate solution, (F) and measure the resulting turbidity in the Exton Junior (or wedge) Scopometer.[5]

That the zinc ferrocyanide turbidity is highly reproducible under these conditions is evident from the following: Sixteen samples of pure glucose solutions, ranging from 0.5 to 10.0 milligrams per c.c., gave an average glucose equivalent[6] per turbidity scale unit of 330 ± 26 (average deviation 8 per cent). Nine samples of urine of known sugar content gave a similar glucose equivalent of 331 ± 19 (average deviation only 5.7 per cent). Twenty specimens of urine run simultaneously with Sumner's dinitrosalicylic acid[7] and the above methods gave an average ratio

$$\frac{\text{Turbidimetric}}{\text{Sumner}} = 1.26 \pm 0.05$$

an average deviation of 4 per cent. Comparison of thirteen samples of urine, run with the yeast, turbidimetric, and Benedict picric acid methods, showed the following average deviations from fermentation results: Sumner's, 0.7; Benedict, 0.75; turbidimetric, 0.6. While not conclusive, the comparison suggests that the new method does not register more nonglucose reducing material than the Sumner and Benedict methods. Applied to blood, the results by this method have been more like Folin's copper method than Benedict's picric acid method.

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*Read before the Sixth Annual Convention of the American Society of Clinical Pathologists in Washington, D. C., May 13, 14, and 16, 1927.

[1]Preliminary report from the laboratory of the Prudential Insurance Co., Newark, N. J.

[2]Lloyd's alkaloidal reagent is shaken in a 20 per cent solution of sodium acid sulphate, filtered by suction, spread thin on glass plates, and dried two hours at 80° C. and reduced pressure.

[3]For urine tests, the reagent consists of 330 grams of potassium ferricyanide and 106 grams of sodium carbonate dissolved in 1000 c.c. of water. For blood, this reagent is diluted five times.

[4]The digestion is preferably done in narrow tubes graduated to hold 20 c.c.

[5]The measurement of the turbidity is very simple by the use of this instrument, and takes but a moment. If the cloud is too dense to read, dilute with 4 per cent ammonia until a reading can be obtained, and include the additional dilution in the calculation.

[6]The number by which the scale reading, multiplied by dilutions, is to be divided to give the glucose in the sample.

[7]Sumner, J. B.: Jour. Biol. Chem., 1925, lxv, 393.

DISCUSSION

Dr. William G. Exton.—This test has the advantage of working over both normal and pathologic ranges of sugar in urine. The colorimetric tests now available are adapted only for the normal range, and when the specimen contains sugar in concentrations above the normal, the tests must be repeated on diluted urines. With Rose's test if the sample is found to contain more sugar than normal, it makes no difference except in calculating the scale readings, and the sugar may be quantitated no matter what the amount.

Dr. G. B. Kramer.—I would like to ask Dr. Rose what advantage this turbidimetric method has for clinical purposes over the method estimating sugar by the polariscope.

Dr. Robert A. Kilduffe.—I would like to say a few words concerning all of the papers thus far presented and to comment upon one angle of the situation. All of us must be impressed with the remarkable advances which have been made in the clinical laboratory along the lines of accuracy and precision. We must remember, however, that unless they are brought to the attention of the clinician and their clinical significance emphasized, they lose much of their value. For example, I have used with great satisfaction Dr. Exton's turbidimetric method of quantitating albumin in the urine, but I frequently encounter the garden variety of clinician who cannot understand so many milligrams of albumin per 100 c.c. until it is translated into "traces" or "moderate amounts."

I don't doubt at all that there will be some of this variety of clinician at the coming American Medical Association meeting who will see Dr. Terry's new stain demonstrated and will get the impression that all that is necessary is to get a bottle of stain and a chunk of tissue and the carcinoma will pop out.

We should endeavor to emphasize consistently and continuously that laboratory tests are not tests for disease but laboratory examinations to determine the reaction of the patient to stimuli, and we should stress the fact that laboratory procedures are methods of examining the patient. And, finally we must educate the clinician to interpret the reports.

Dr. Wm. G. Exton.—As to the polariscope I may say that it makes a great deal of difference which polariscope is used and also who uses the paliriscope. The polariscopes and saccharimeters usually seen in books on clinical pathology and in clinical laboratories are almost always of an inferior and inadequate type and insensitive as compared with our chemical methods. Some of them that I have seen will hardly detect the difference between none and 1 per cent of sugar. I fear that many who use these instruments in their routine work have very illusory ideas as to the accuracy of the results they obtain, especially with short tubes and when the preliminary treatment is insufficient to properly clarify and exclude other rotatory substances which occur in urine. A good polariscope with accessories costs something like a thousand dollars and demands not only skill and experience of the observer but also great care in controlling temperature, wave length and the other physical factors involved which make the technic very time consuming. Notwithstanding its perfection as an optical method I, therefore, regard the polariscope as being unsuited for routine urinalysis.

TRANSACTIONS

Minutes of the Sixth Annual Convention, American Society of Clinical Pathologists—Washington, D. C.

THE proceedings were held in the ballroom of the New Willard Hotel, Washington, D. C., May 13, 14, and 16, 1927. Convention called to order by President, Wm. G. Exton, Newark, New Jersey.

and Dr. Robert A. Keilty, Washington, D. C., proposed changes in the constitution and by-laws to be adopted at the Executive Session.

Dr. C. E. Roderick, Battle Creek, Michigan, moved that a vote of thanks be directed to Dr. Wm. H. Moursund and the Dallas Committee for their entertainment of the Fifth Annual Convention. Motion carried.

There being no further business before the session, the remaining time was devoted to the reading of papers on the regular scientific program as follows:

"A Study of the Micro-Kahn Test in Syphilis" by Dr. Robert A. Kilduffe, Atlantic City, New Jersey.

"The Microscopic Kahn Reaction" by Dr. Francis B. Johnson, Charleston, South Carolina.

"Further Studies of the Kolmer and Kahn Tests" by Dr. C. E. Roderick, Battle Creek, Michigan.

Symposium of Kahn test discussed by Dr. R. L. Kahn, Lansing, Michigan, Dr. B. S. Kline, Cleveland, Ohio, Dr. H. A. Heise, Uniontown, Pennsylvania, Dr. Robert A. Kilduffe, Atlantic City, New Jersey, Dr. Robert A. Keilty, Washington, D. C., Dr. Francis B. Johnson, Charleston, South Carolina, and Dr. R. L. Kahn (closing).

"Fatalities Following the Use of Arsphenamine With Report of Autopsy" by Dr. Ernest Scott, and Dr. R. A. Moore, Columbus, Ohio. Read by title.

"Brain Structure Changes After Treatment in General Paralysis" by Dr. A. M. P. Saunders, Dunning, Illinois. Discussion by Dr. G. B. Kramer, Youngstown, Ohio, and Dr. A. M. P. Saunders (closing).

"The Use of Injection Methods in Pathology" by Dr. Ernest Scott, and Dr. R. A. Moore, Columbus, Ohio. No discussion.

Friday, May 13, 1927, 2 P.M.

"Blood Picture of Purpura" by Dr. Nathan Rosenthal, New York City. Discussion by Dr. Reuben Ottenberg, New York City, Dr. Reed Rockwood, Baltimore Maryland, and Dr. Nathan Rosenthal (closing).

"Anemia as a Factor in the Test of the Rate of Sedimentation of the Erythrocytes" by Dr. Roger S. Hubbard, Clifton Springs, New York (by invitation).

"Studies of Sedimentation of Erythrocytes" by Dr. A. H. Sanford, and Dr. H. F. Hunt, Rochester, Minnesota. Both papers discussed by Dr. Carl Spohr, Columbus, Ohio, Dr. Asher Yaguda, Newark, New Jersey, Dr. Herman Sharlit, New York City, Dr. H. R. Brown, Rochester, New York, Dr. W. G. Exton, Newark, New Jersey, Dr. Roger S. Hubbard, Clifton Springs, New York, Dr. A. H. Sanford, Rochester, Minnesota, and Dr. G. B. Kramer, Youngstown, Ohio.

"Differential Blood Counts: A Comparison of the Accuracy Obtained by Various Methods" by Dr. Dean N. Beacom, Denver, Colorado. Discussion by Dr. A. H. Sanford, Rochester, Minnesota, Dr. Robert A. Keilty, Washington, D. C., Dr. F. B. Johnson, Charleston, South Carolina, Dr. Nathan Rosenthal, New York City, Dr. Dean N. Beacom (closing).

"Ovarian Function: Its Influence on the Concentration of Calcium in Blood" by Dr. Herman Sharlit, and Dr. Wm. G. Lyle, New York City. No discussion.

"Purpuric Smallpox, Review of Recent Studies" by Kano Ikeda, St. Paul, Minnesota. No discussion.

Friday, May 13, 1927, 8 P.M.

"A Key to the Diagnosis of Neoplasms" by Dr. Wm. Carpenter MacCarty, Rochester, Minnesota. Discussed by Dr. B. T. Terry, Rochester, Minnesota.

"Rapid Methods of Examining Tissue Microscopically Without a Microtome" by Dr. B. T. Terry, Rochester, Minnesota. Discussed by Dr. Wm. Carpenter MacCarty, Rochester, Minnesota, Dr. Harold G. Palmer, Philadelphia, Pennsylvania, and Dr. Philip Hillkowitz, Denver, Colorado.

"The Present State of Our Knowledge of Gingivitis" by Dr. Robert A. Keilty, Washington, D. C. Discussed by Dr. Philip Hillkowitz, Denver, Colorado and Dr. Robert A. Keilty (closing).

"The Etiologic and Specific Relationship of Foci of Infection to Certain Organic Lesions: A Postmortem Study" by Dr. A. S. Giordano, South Bend, Indiana. No discussion.

"The Budding of Ameba" by Dr. L. H. Prince, Washington, D. C., and Dr. T. H. T. Wight, Palo Alto, California (by invitation). No discussion.

Saturday, May 14, 1927, 9 A.M.

"Accuracy and Precision in Clinical Pathology" by Dr. P. V. Wells, Newark, New Jersey (by invitation). Discussed by Dr. Herman Sharlit, New York City and Dr. P. V. Wells (closing).

"New Clinical Methods for Measuring Color and Turbidity as Applied in the Junior Scopometer" by Dr. Wm. G. Exton, Newark, New Jersey. No discussion.

"A Turbidimetric Method for Sugar" by Dr. Anton R. Rose, Newark, New Jersey (by invitation). Discussed by Dr. Wm. G. Exton, Newark, New Jersey, Dr. G. B. Kramer, Youngstown, Ohio, and Dr. R. A. Kilduffe, Atlantic City, New Jersey.

"Sugars in Normal Urine" by Dr. Isadore Greenwald, New York City (by invitation). Discussed by Dr. A. H. Sanford, Rochester, Minnesota, and Dr. Wm. G. Exton, Newark, New Jersey.

"Occurrence of Lipoids in Urine and Their Diagnostic Importance" by Dr. E. L. Miloslavich, Milwaukee, Wisconsin. Discussion by Dr. W. G. Exton, Newark, New Jersey, Dr. Mortimer Herzberg, Cincinnati, Ohio, Dr. E. L. Miloslavich (closing), and Dr. Wm. G. Exton, Newark, New Jersey.

"The Comparative Diagnostic Value of the Levinson Test and the Glucose Content of the Cerebrospinal Fluid in Tuberculous Meningitis" by Dr. A. S. Giordano, South Bend, Indiana.

Saturday, May 14, 1927, 2 P.M.

"Pathology of Intestinal Tuberculosis" by Alfred Blumberg, Oteen, North Carolina. No discussion.

"The Cultivation of Tubercle Bacilli" by Dr. H. J. Corper and Dr. Nao Uyei, Denver, Colorado. Discussed by Dr. Robert A. Keilty, Washington, D. C., Dr. C. E. Royce, Bethlehem, Pennsylvania, Dr. Frank W. Hartman, Detroit, Michigan, Dr. George Ives, St. Louis, Missouri, and Dr. H. J. Corper (closing).

"Pathologic Laboratory Examinations for the Dentist" by Dr. Chas. G. Darlington, New York City. Discussed by Dr. Robert A. Keilty, Washington, D. C., Dr. Wm. G. Exton, Newark, New Jersey, and Dr. Chas. G. Darlington (closing).

"A Modification of the Technic of the Wassermann Test" by L. H. Cornwall, Dr. D. Groszberg, and Blanche C. Taylor, New York City. Discussion by Dr. H. R. Brown, Rochester, New York, and Dr. D. Groszberg (closing).

Saturday, May 14, 1927, 7 P.M.

The sixth annual banquet was held in the Willard Room of the New Willard Hotel, Saturday evening, May 14, 1927, at 9 P.M. President Wm. G. Exton gave an address, "The Relation of Clinical Pathology to Preclinical Medicine." Other addresses of the evening

were as follows: "The Relation and Responsibilities of the Clinical Pathologist to the Hospital Standardization Movement" by Dr. M. T. MacEachern, American College of Surgeons, Chicago.

Remarks by Dr. George K. Burgess, Director of the Bureau of Standards, Washington, D. C.

Remarks by Dr. George S. McCoy, Director Hygienic Laboratory, Washington, D. C.

Remarks by Rear Admiral E. R. Stitt, Navy Department, Washington, D. C.

Monday, May 16, 1927, 9 A.M.

The business session was called to order by Dr. Wm. G. Exton, President. The reading of the minutes of the last meeting was dispensed with, these having been published.

The report of the Executive Committee was given by Dr. Philip Hillkowitz, Chairman, who read the financial report and stated that the books of the Secretary were found to be correct. This report was accepted by motion of the Society.

The matter of the publication of an official organ was taken up and the Executive Committee reported progress. Upon motion the matter was re-referred to the Executive Committee for consideration and to report back to the Society. Discussion by Dr. A. H. Sanford, Dr. H. G. Palmer, Dr. Mortimer Herzberg, Dr. Wm. G. Exton, and Dr. Frank W. Hartman.

The next matter to be reported on by the Executive Committee was the State Laboratory question. Dr. Hillkowitz read a summary of a survey of the opinions of the membership on this question. Discussion by Dr. Thomas F. Walker, Great Falls, Montana, Dr. Mortimer Herzberg, Cincinnati, Dr. Frederic E. Sondern, New York City, Dr. Wm. Carpenter MacCarty, Rochester, Minnesota, Dr. Robert A. Kilduffe, Atlantic City, Dr. H. R. Brown, Rochester, New York, Dr. J. J. Moore, Chicago, Dr. George Ives, St. Louis, and Dr. B. W. Rhamy, Fort Wayne, Indiana. Motion was carried that the activities of the Society for the present, at least, be confined to trying to educate the medical profession to the value of laboratory methods.

The report of the Committee on Exhibits was given by Dr. A. H. Schade, Chairman. Upon motion of the Society it was accepted.

Dr. Frederic E. Sondern, Chairman, gave the report of the Committee on Public Relations. Report accepted.

The report of the Committee on Research was presented by Dr. H. J. Corper, Chairman. Report was accepted by the Society.

Under this report was taken up the matter of standardizing the nomenclatures for blood typing, using the Von Dungern and Hirschfeld method as suggested by Dr. Reuben Ottenberg, New York City. Discussed by Dr. F. W. Hartman, Detroit, Dr. F. E. Sondern, New York City, Dr. A. J. Casselman, Camden, New Jersey. Upon motion the matter was referred to a Committee to be appointed by the President, with the power to act for the Society.

The report of the Publication Committee was made by its Chairman, Dr. John A. Kolmer, Philadelphia. The matter of a book of methods being published under the auspices of the Society was discussed by Dr. Wm. Carpenter MacCarty, Rochester, Minn., Dr. A. H. Sanford, Rochester, Dr. Wm. G. Exton, Newark, Dr. A. G. Sandblad, McKeesport, Pennsylvania, Dr. H. J. Corper, Denver, and Dr. Herman Spitz, Nashville, Tennessee. Report accepted and motion carried that Dr. Kolmer be continued as Chairman of the Publication Committee; second, that the committee appoint the editors and sub-editors or associate editors; third, that work be begun at once simultaneously on different fields included in the scope of the book.

Report of the Service Bureau Committee was made by Dr. R. A. Kilduffe, Chairman. Dr. Kilduffe reported progress. Accepted by Society.

The report of the Committee on the Registration of Technicians was presented by Dr. Kano Ikeda, a member of the Committee. Report was accepted and referred to the Committee.

Upon recommendation of the Board of Censors the following were elected to active membership: Dr. Dean N. Beacom, Denver, Colorado, Dr. Lester Neuman, Washington, D. C., Dr. Alvin G. Foord, Buffalo, New York, Dr. Wm. L. C. Spaeth, Philadelphia, Penn-

sylvania, Dr. Everett L. Bishop, Atlanta, Georgia, Dr. Garner Scullard, Pittsburgh, Pennsylvania, Dr. Oscar B. Hunter, Washington, D. C., Dr. Regina Cook Beck, Richmond, Virginia, Dr. Iva C. Youmans, Miami, Florida, Dr. Gordon Priestman, Long Island, New York, Dr. Manuel G. Giehner, Baltimore, Maryland, Dr. John W. Gray, Newark, New Jersey, and Dr. W. G. Gamble, Jr., Charleston, South Carolina.

To associate membership: Dr. M. N. Richter, New York City, and Dr. W. N. Rogers, Trenton, New Jersey.

Considerable discussion took place on the subject of admitting Doctors of veterinary medicine as associate members, and a motion was carried to refer the matter to the Executive Committee.

Three changes in the constitution and by-laws were then read and adopted by the Society. To wit:

1. "A Nominating Committee of three shall be appointed by the President at the opening session of the meeting, whose duty it shall be to prepare a list of nominees for the various offices for balloting by the Secretary. Additional nominations may be made from the floor."

2. "The Secretary-Treasurer shall serve a term of three years."

3. Delete "Appointment of Committees" under "Order of Business for Executive Session."

The election of officers resulted as follows: Dr. A. H. Sanford, President, Rochester, Minnesota; Dr. F. W. Hartman, President-Elect, Detroit, Michigan; Dr. H. J. Nichols, Vice-President, Quarry Heights, Panama; Dr. Ward Burdick, Secretary-Treasurer, Denver, Colorado.

Executive Committee, Dr. Frederic E. Sondern, New York City and Dr. Wm. G. Exton, Newark, New Jersey.

Board of Censors: Dr. Reuben Ottenberg, New York City and Dr. C. H. Manlove, Portland, Oregon.

The meeting was adjourned.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

LABORATORY TECHNIC

CANCER: The Serodiagnosis of Cancer, Hartmann, H. Bull. de l'Acad. de med., April, 1926, xcv, 412.

Technic: The amount of albumin in the serum is first determined by a refractometer. If the serum is hyperalbuminous sufficient salt solution is added to bring it to a normal albuminous composition. If it is hypoalbuminous it is concentrated by heating.

The following solutions are used:

1. Solution of nitric acid (1 c.c. of pure nitric acid at 36° Baumé; 100 c.c. physiologic salt solution).

2. Iodo-iodide reagent (bi-sublimed iodine 1 gm. potassium iodide 2 gm., distilled water 210 gm.).

Three c.c. of the nitric acid reagent are placed in a test tube and 0.5 c.c. of serum is added and shaken. Five-tenths c.c. of the iodine reagent is then carefully added to the slight foam on the surface of the mixture. A slight precipitate forms at the junction of the two fluids. The thumb is placed over the mouth of the tube and it is shaken slightly. The precipitate gradually settles to the bottom of the tube and dissolves. Then the tube is inversed several times.

This process is repeated after the addition of another 0.5 c.c. of the iodine reagent. In certain very positive cases the precipitate formed does not completely dissolve.

After a further addition of 0.3 c.c. of the iodine reagent, the same procedure is followed. When all the precipitate has fallen to the bottom of the tube, the tube is inverted and is then placed right side up. When all air bubbles have ascended to the surface the contents are examined by transparency.

If the fluid is clear the reaction is negative. A slight flocculation is the threshold of a positive reaction, if cloudy, the reaction is positive.

In 145 cases of cancer there were 131 positive reactions. In 4 of the 14 negative cases the cancer had been irradiated before the serum was taken and in one other case the diagnosis of cancer of the tongue was not confirmed by biopsy.

Among 55 controls there were 47 negative reactions.

As a whole, this reaction has an equal value for the diagnosis of cancer as the Bordet-Wassermann reaction for the diagnosis of syphilis.

ACETONURIA: On a New Method of Estimation of Acetone and Its Application to the Problem of Normal Acetonuria, Fleury, P., and Awad, I. J. Compt. rend. Soc. de biol., March 12, 1926, xciv, 570.

Reagent:

Solution A

Mercury bichloride	13.55
Potassium iodide q.s.	36.00
Distilled water q.s.ad.	500.00

Solution B

Pure soda	27.00
Distilled water q.s.ad.	100.00

Take 21 c.c. of A and 9 c.c. of B.

1. Distillation of the urine. One hundred c.c. (or less if it contains much acetone) of urine, acidified by 1 c.c. of phosphoric acid, is boiled in a distilling apparatus provided with a rectifying tube and a current of steam is passed through it. The distillate is received with the precautions necessary to avoid any loss by vaporization.

If there is 0.50 or less acetone per liter, the first 10 or 15 c.c. of distillate will contain all the acetone. If there are greater quantities of acetone, 20 to 25 c.c. of distillate will suffice.

2. Determination of the acetone in the distillate. To 5 c.c. or 10 c.c. of the distillate containing a maximum of 5 mg. of acetone, add 30 c.c. of the reagent. After coagulating of the precipitate (fifteen to twenty minutes) centrifuge and decant, then, the tube being placed in cold water, treat the precipitate with 1 c.c. of hydrochloric acid (sp. gr. 1.17) diluted with an equal volume of water and 5 c.c. of a 20 per cent solution of potassium iodide. Filter through a very small filter without folds to separate the reduced mercury and receive the filtrate in a tube immersed in cold water. Wash the filter three times with water using 2 c.c. each time. To the mixture of filtrate and wash waters, add 10 c.c. of deci-normal iodine and 10 c.c. of a 27 per cent solution of sodium hydrate. After ten minutes acidify with 15 c.c. of hydrochloric acid (sp. gr. 1.17) diluted to $\frac{1}{10}$ its volume and titrate the free iodine with a deci-normal solution of sodium hyposulphite. Calculate the result. Each cubic centimeter of iodine solution consumed corresponds to 0.967 mg. of acetone.

Results obtained with normal urine. Normal urine was examined by three different methods and the results showed that, with the exception of one case, the acetone amounted to just about 1 mg. per liter.

BLOOD: The Transformation of Monocytes into Fibroblasts Through the Action of Raus Virus, Carrel, A., and Ebeling, A. H. Jour. Exper. Med., April, 1926, xliii, No. 4, p. 461.

It is well known that one of the more important cultural characteristics of monocytes is their inability to form a tissue. When free to move in the medium, they always place themselves at a certain distance from one another. If they are packed together by centrifugation and embedded in a film of plasma, they migrate rapidly from the coagulum. When migration is impossible, they die. Fibroblasts, on the contrary, always live as a tissue. When they are in close contact, they multiply actively without scattering over the coagulum. They remain packed together. The peripheral cells of the growing colony are generally in contact with one another, or are united by their processes. It is important to observe that the transformation of monocytes into fibroblasts generally occurs when, on account of some change in the medium, the life of the monocytes has become impossible.

The monocytes transform themselves into cells capable of living under the conditions present in the culture. The metamorphosis of a monocyte into a fibroblast displays the characteristic of an adaptive change which may automatically be produced by substances set free by the monocytes themselves under certain conditions.

The phenomenon that occurs in cultures of monocytes inoculated with Rous virus probably has the same significance. When an area of digestion occurs in the coagulum, masses of necrotic tissue appear along its edges. Around these masses, the fibroblastic forms develop. The monocytes that are highly susceptible to Rous virus become transformed into fibroblasts that are not sensitive to the virus, and that are not even a favorable medium for the growth of the virus. It seems as if there were a tendency for a susceptible cell to transform itself into an immune cell. This phenomenon could be considered as an expression of the general property with which all living organisms or chemical systems are endowed, that of opposing the action of a disturbing factor. But the real significance of these facts cannot be understood until we know whether the transformation of monocytes into fibroblasts is reversible.

In normal cultures, the transformation of monocytes into fibroblasts generally occurred when cells became packed together through some mechanical factors that prevented their

free migration and determined their accumulation. Various modifications of the medium, the addition of dead tissue, and of trypsin or the products of trypsin digestion, failed to bring about the transformation. The inoculation of cultures of monocytes with filtered extract of Rous sarcome frequently determined the appearance of fibroblasts. The first change undergone by the monocytes cultivated in vitro was a large increase in their size. Later, the giant monocytes became transformed into cells that did not differ essentially from those that grow from a fragment of adult connective tissue.

BLOOD: Chemical Differentiation of Races, Manoiloff, E. O. München. med. Wchnschr., 1925, lxxii, 2186.

To 3 c.c. of a 3 to 5 per cent emulsion of cells obtained from the blood clot add one drop of 1 per cent alcoholic solution of methylene blue. Mix and add 5 drops of 1 per cent alcoholic cresyl violet and again mix.

Add 3 drops of 0.5 to 1 per cent silver nitrate, stir, and add 1 drop of 40 per cent HCl.

Stir well and add 3 to 5 or more drops of freshly prepared aqueous 1 per cent potassium permanganate, the amount depending upon the quality of the dyes.

Using this method Manoiloff claims that chemical differentiation of races is possible, reporting 187 correct results in 202 tests.

Cresyl violet disappears in Jewish blood which becomes blue, while the fluid stays bluish red in Russian blood. The children of mixed marriages (father Russian, mother Jewish, Polish or Armenian) gave a faster reaction than pure Russians. Intermarriage between Russians, Germans, and yellow races evidenced no difference from Russians.

The reaction is explained on the grounds of greater speed of oxidation of Jewish blood.

CANCER: An Analysis of the Botelho Serum Test for Cancer, Baty, J. M., and Greene, J. A. Arch. Path. and Lab. Med., August, 1926, ii, No. 2, p. 217.

Report of a series of tests upon 79 patients with cancer, 62 cases of various diseases, and 4 normal persons. The diagnosis of cancer was confirmed microscopically in 65.8 per cent of the cancer cases.

The test was made in accordance with directions received from Dr. Botelho directly as follows:

1. The serum is removed from the ice box, if it has been stored, and allowed to attain room temperature.

2. Three c.c. of the nitric acid solution is placed in a test tube 10 cm. in length with a bore of about 1.5 cm.

3. To this is added 0.5 c.c. of the serum (corrected to 7.8 to 8 gm. protein per 100 c.c.). This is shaken until the serum and nitric acid solution are thoroughly mixed.

4. Then 0.5 c.c. of the iodine solution is added. A flocculent precipitate forms at the junction of the iodine solution and the nitric acid-serum mixture. The tube is shaken very gently at first, and then gradually more violently until the precipitate has been completely dissolved.

5. Then 0.5 c.c. of the iodine solution is added and shaken as before.

6. To this 0.3 c.c. of the iodine solution is added and shaken gently until the precipitate is dispersed throughout the tube. The procedure is then completed. If the contents of the tube remain perfectly clear, the reaction is negative. If there is definite cloudiness after 1.3 c.c. of the iodine solution have been added, the reaction is considered positive. Observations are best made in artificial light, using a 25-watt electric light bulb as a background. If the reaction is negative, the degree of negativity should be determined by the further addition of iodine solution, 0.2 c.c. at a time, until a permanent precipitate appears.

The authors conclude that Botelho's reaction is of no value in the diagnosis of cancer.

It depends, like many similar tests, on some change in the physical state of the blood colloids, and may give positive results on serums from patients suffering from any disease condition in which there is such a change.

LACTOSE FERMENTING BACTERIA: Studies on Lactose Fermenting Bacteria, Berry, F., and Ey, L. F. Am. Jour. Pub. Health, May, 1926, xvi, No. 5, p. 491.

A total of 287 well waters varying in sanitary quality, as determined by careful survey of the sources, from "good" to "poor," were examined for colon bacilli with particular reference to the value of the methyl red and Voges-Proskauer reactions. A majority of these wells (198) were located in a village and therefore were probably subject to more direct contamination with human discharges because of the proximity of outdoor toilets on the same or neighboring lots. The remainder of the wells (89) were in a rural district where the danger of contamination with human discharges would probably be less but the danger from animal discharges greater than in the village wells. The percentages of methyl red positive and Voges-Proskauer negative cultures in the urban and rural wells were practically the same. It is understood, of course, that no claim has ever been made that these two tests would distinguish between human and animal discharges, but it was thought that possibly some difference might be found in the relative numbers of fecal and soil types in the two groups of wells.

There was an appreciable difference in the percentage of so-called "fecal" types in the "good" and "poor" wells in each series. This difference was apparent to about the same degree on the basis of the methyl red, the Voges-Proskauer, and the indol test, and it agreed rather closely with the difference in percentage of confirmed colon tests in the respective wells. It is difficult to place a strict interpretation on these figures because there is no established scale of expected frequency of the fecal and soil types as we progress from a grossly polluted water to one of excellent quality. The important point is whether too many "good" wells show fecal types as established by the differential tests. The percentage of fecal types in the "poor" wells is of minor importance, for these supplies would be condemned on the sanitary survey alone.

The striking feature of the results in both rural and urban wells was the number of "MR+ VP–" cultures in the "good" wells, and particularly in the "very good" drilled wells. It is scarcely conceivable that 70 per cent of the colon group bacteria in these supplies are actually of fecal origin. The only satisfactory explanation which conforms with our conception of safe well water, as determined by field sanitary survey, is that many of the cultures constituting this 70 per cent are soil types and are therefore of slight sanitary significance. This confirms Koser's conclusion based on his sanitary survey of the waters he examined and on comparative tests with his citrate medium.

Without attempting to attach too much importance to the indol results in this series it is perhaps worth noting that the lower percentage of indol positive cultures correlates more closely with the sanitary survey than does either the methyl red or Voges-Proskauer reaction.

Conclusion.—Absence of methyl red positive and Voges-Proskauer negative members of the colon group in ground waters of good sanitary quality is significant, but the presence of this type in such waters cannot be regarded as conclusive evidence of fecal contamination. These differential tests, therefore, have very limited value in the analysis of well waters.

TUBERCULOSIS: Tubercle Bacilli in Tuberculosis Pus, Gardner, A. D. Lancet, June 5, 1926, 1090.

Gardner believes that the frequent failure to find tubercle bacilli in pus is due to unsuitable counter stains and that if a yellow counter stain (half-saturated aqueous picric acid) is used the organism may be found in 90 per cent of cases.

PHAGOCYTOSIS: Type of Phagocytic Cell and Its Relative Proportions in Human Bone Marrow and Spleen, as Identified by Supravital Technic, with Special Reference to Pernicious Anemia, Doan, C. A. Jour. Exper. Med., March, 1926, xliii, No. 3, p. 289.

In general, there is a reversal of the normal in the ratio of clasmatocytes in the spleen to clasmatocytes in the bone marrow in pernicious anemia, with a marked tendency toward the phagocytosis of young, immature, nucleated red blood cells in the bone marrow. The peripheral blood picture suggests that these cells had never been in circulation. The observations made do not indicate that the spleen takes any directly active part in an increased destruction of blood in pernicious anemia.

GRANULOMA INGUINALE: The Etiology of Granuloma Inguinale, McIntosh, J. A. Jour. Am. Med. Assn., September 25, 1926, lxxxvii, 996.

This study of the etiology of granuloma inguinale was undertaken for the purpose of determining the nature, transmissibility and differential diagnosis of the causative agent and the period of the infection.

Fifteen spontaneous cases furnished the material for the study reported, typical Donovan bodies being found by direct smear in fourteen cases and pure cultures being grown from eight cases.

Biopsy material was fixed in equal parts of 10 per cent formaldehyde and 2.5 per cent aqueous potassium bichromate and stained with hematoxylin and eosin. There are four camera lucida drawings of the pictures seen.

The formol-gel test first described by the author in this disease (Memphis Med. Jour., 1925, ii, 139) was positive in fourteen cases.

The technic is as follows:

To 1 c.c. of patient's blood serum is added 1 drop of 37 per cent formaldehyde, and mixed thoroughly. Evaporation is prevented and the mixture allowed to stand for forty-eight hours. If positive, there is opacity and gelling of the serum; in some cases the gelling occurs in less than ten minutes.

The organism was found to grow best on Sabourand's medium.

The cultivated organism was pleomorphic, coccoid to bacillary in shape, nonmotile, nonsporulating, from 0.5 to 2 microns in diameter, Gram-negative, and stained readily with Wright's stain. Primary cultures, vitally stained with brilliant cresyl blue, suspended in 0.85 per cent saline solution, showed zooglea matrix, embedded coccoid forms and numerous, unequal tetrad grouping. The impression was obtained, from a study of the vitally stained organisms, that many of the coccoid bodies multiplied in a line assuming a bacillary form, some of which would swell into large, oval bodies 3 or 4 microns in diameter. The coccoid form embedded in gelatinous nongranular matrix was the most constant, and those just mentioned were inconstant findings. The anaerobically grown organism was generally larger than the one grown aerobically. The organism grown on Lemco medium was oval to bacillary in form and showed the most marked variation in size. Threadlike filaments were observed in cultures on this medium.

Two instances of successful inoculation in the human being are reported.

The study reported in this paper tends to prove that the Donovan body is the cause of granuloma inguinale by fulfilling the generally accepted criteria of specificity first stated by Koch. First, the organism was demonstrated in the lesion in fourteen out of fifteen spontaneous cases. Second, it was obtained in pure culture in eight of these fifteen cases. Third, a tissue graft from a spontaneous case in which Donovan bodies were demonstrated and from which they were isolated in pure culture reproduced the disease in an individual not previously exposed in any way to the possibility of spontaneously contracting this disease. Fourth, pure culture of the Donovan body was again obtained from this experimental lesion. And, further, antibody production against the cultivated Donovan body was demonstrated by the presence of agglutinins, precipitins, globulin changes and skin sensitiveness in the spontaneous and experimental cases.

This is the first reported instance of successful experimental transmission of granuloma inguinale from one individual to another.

The data support the belief that the Donovan body is the cause of granuloma inguinale and that it is a bacterium unrelated to the Friedlander group of organisms.

Repeated exposure of normal individuals through coitus to those suffering with granuloma inguinale without their contracting the disease suggests that an actual break in the skin surface is necessary for successful inoculation or that the organism is infective only for susceptible individuals.

The growth of the Donovan body in rather low dilutions of antimony and potassium tartrate may indicate that the action of the drug within the body may be indirect rather than direct.

The blood globulins are disturbed either qualitatively or quantitatively, or both, in granuloma inguinale. The disturbance may be determined by the formol-gel test.

Brilliant cresyl blue dye dissolved in physiologic sodium chloride solution is a rapid and satisfactory stain for Donovan bodies when used without fixation and dehydration.

Gentian violet given intravenously was found to inhibit the progress of the disease. It is much less effective however, than antimony and potassium tartrate.

EPIDEMIC HICCUP: Further Studies on the Etiology of Epidemic Hiccup (Singultus) and Its Relation to Encephalitis, Rosenow, E. C. Arch. Neurol. and Psychiat., June, 1926, xv, 712.

The results of previous studies have been verified in detail in two additional series of cases. A streptococcus, alike in morphology and with similar cultural characters and having similar immunologic reactions, was isolated from the infection atrium in twenty additional cases of epidemic hiccup, and with each strain, spasms of the diaphragm or other muscles were produced in animals. The organism was isolated from these animals and characteristic symptoms again induced on inoculation. It was demonstrated in the lesions, and proved absent elsewhere, by microscopic examination of sections. Similar experiments, made with streptococci from cases of poliomyelitis and other diseases of the nervous system, from poliomyelitis contacts, from normal controls and from patients that had recovered from hiccup, gave strikingly different results. The possibility of an accompanying filterable virus (in the usual meaning of that term) being the cause of the spasms was excluded by the successful reproduction of characteristic symptoms with some of the strains after many rapidly made subcultures, with the dead streptococci, and with filtrates of active cultures, the symptoms in the case of filtrates beginning in from one to three hours after injection.

Positive results were obtained by methods in which the conditions in patients were closely simulated, as well as by intracerebral inoculation of cultures, in some instances in more than one species of animal. Packing of the nose with gauze soaked in cultures sufficed to provoke spasms of the diaphragm and other muscles. Of the common laboratory animals, rabbits were found most susceptible, but similar results were sometimes obtained in guinea pigs and monkeys (Macacus rhesus).

The streptococcus produces in the throat of the patient, as in cultures, a filtrable substance which has the power of inciting spasms of the diaphragm and other muscles. The type of disease and the lesions induced were in many respects similar to those noted in the spontaneous disease. The spasms of the diaphragm have been seen through an incision in the abdominal wall made under local anesthesia, and by means of the fluoroscope. They were usually severe, and continued for hours and sometimes days. In the animals, as in man, synchronous spasms of the abdominal muscles frequently occurred. During the severe spasms audible hiccup was frequently noted in rabbits and monkeys. The conclusion that epidemic hiccup is due to a streptococcus (Streptococcus singultis) having peculiar neurotropic properties seems warranted.

The close relationship believed to exist between epidemic hiccup and epidemic encephalitis, and indicated by epidemiologic findings, receives much support in these experiments. The symptoms and lesions in the animals that succumbed were similar to those in fatal cases of hiccup reported in the literature. In some instances, especially after one or more passages through animals, and after many subcultures, the streptococcus from cases of hiccup no longer produced spasms of the diaphragm but instead lethargic or other forms of encephalitis. Spasmodic torticollis, which developed as a late manifestation, was as common in the hiccup series as in the encephalitis experiments. The strains from the two diseases, culturally indistinguishable, are cross agglutinated and the cleared nasopharyngeal extracts in a sodium chloride solution cross precipitated by the respective antistreptococcus serums; and the encephalitis hyperimmune horse serum had marked curative effects in animals having spasms of the diaphragm following injection of the streptococcus from cases of hiccup. The results from serum and vaccine treatment of cases, while striking, are far too few to be conclusive. The facts, however, that the serum had a marked curative effect on animals under controlled conditions, that normal horse serum had no effect and that active immunization afforded protection for rabbits injected with the hiccup streptococcus, afford a rational basis for the attempts at passive and active immunization.

DIABETES MELLITUS: The van den Bergh Reaction in Diabetes Mellitus, Rabinowitch, I. M. Frith, A.B., and Wylde, K. Brit. Jour. Exper. Med., June, 1926, vii, 155.

The purpose of this communication is to record the observations which were made with reference to the van den Bergh reaction in 130 cases of diabetes mellitus. The results obtained further emphasize the relation which exists between diseases of the biliary passages and diabetes, and also demonstrate the value of this test in the management of the latter disease.

The van den Bergh reaction, known to be a sensitive test for the detection of excess bilirubinemia, was applied to a group of individuals with biliary disease.

The incidence of positive reactions found suggested that the liver escapes injury in a minority of such cases only.

Because of this finding and the known relationship between disease of the biliary passages and diabetes, the reaction was observed in a series of diabetics.

Of a group of 130 diabetics, positive van den Bergh reactions were found in 34 cases—an incidence of 26 per cent. This incidence agrees closely with that found in cholelithiasis in the 68 cases of diabetes observed by Jones, Castle, Mulholland and Bailey.

Since gall bladder disease is proved to be an important etiologic factor in the production of diabetes, and in view of the fact that as compared with diabetes, it can be more simply, and certainly more satisfactorily treated, the necessity of a simple test for its detection is obvious. The van den Bergh reaction is of value for this purpose. The routine performance of this test, may, therefore, be of assistance in the prevention of diabetes.

As with all other laboratory tests, it is only of value when properly interpreted. For the latter purpose, correlation with the clinical picture is essential in order to exclude conditions other than gall bladder disease and pancreatitis (hemolytic processes, heart failure, etc.) which may cause positive reactions.

PNEUMOCOCCUS: Immunity to Pneumococcus in Rats Produced by Feeding Them the Germ, Ross, V. Jour. Immunol., September, 1926, xii, No. 3, p. 237.

Rats which are fed the living pneumococci from 50 c.c. culture per day are considerably more resistant to intraperitoneal injections of the same organism than are untreated control animals.

The degree of protection is as good, although not so regular as, that previously reported as having been obtained when the tissues of animals killed by pneumococcus were fed.

Rats which are fed the heat killed pneumococci (80° C. two hours) in the same quantities show a decidedly inferior degree of immunity.

In a second paper (Immunity to Pneumococcus Produced in Rats by Feeding Tissues of Animals Killed by the Same Organism, Ross, V., Jour. Immunol., September, 1926, xxi, No. 3, p. 219) it was found that rats fed on tissues of animals killed by pneumococcus Type I show an increased resistance to intraperitoneal injections of the same germ.

The protection seems to be type specific.

Protective substances exist in the sera of the immunized animals.

CEREBROSPINAL FLUID: Is the Sugar Content of the Cerebrospinal Fluid Increased in Pregnancy and Myoma, Wellmuth, K. Deutsch. med. Wchnschr., May 7, 1926, lii, 735.

In 45 cases of normal pregnancy; 45 normal nonpregnant women, and 25 cases of myoma, normal cerebrospinal sugar values (0.005 to 0.0075), were found with the Folin-Wu technic.

In 5 cases of myoma the values were at the upper limit of normal in both blood and spinal fluid. In these cases there had been severe hemorrhage and in view of the fact that a relative increase in blood sugar has been observed after venesection, this is explainable by the hemorrhage.

The results do not confirm the observations of Vogt that cerebrospinal sugar is increased in myoma and pregnancy.

BLOOD CALCIUM: The Calcium Content of the Blood in Leprosy, Concepcion, I., and Salcedo, J. Jour. P. I. Med. Assn., May, 1926, 151.

Determination of the calcium content of the blood of patients in various stages and of different forms of leprosy under treatment revealed that the values for calcium in all the cases examined, except paroled and suspect cases, were within normal limits. The average calcium found in cases of less than one year duration was much less than in those of more than one year duration. No marked difference was noticed in the two types of leprosy. The slight increase in the calcium content, observed in cases that have recovered and have been paroled (Nos. 30, 31, and 32) was not due to the effect of the injection of ethyl ester.

NEPHRITIS IN PREGNANCY: A Clinical Study of Nephritis in Cases of Pregnancy, Rockwood, R., Mussey, R. D., and Keith, N. M. Surg., Gynec. and Obst., March, 1926, 342.

A study of one hundred cases in which renal damage occurred during pregnancy. Only fifty-seven cases in which there was hypertension, and nephritis are discussed at length, the remaining 43 cases of pyelitis or pyelonephritis being omitted.

Many of the toxemias of pregnancy are associated with nephritis and can be classified as are other types of nephritis, not necessarily occurring in pregnancy. The classification of Volhard and Fahr is followed.

The course of fifty-seven cases during pregnancy is followed, together with the fate of the mother and child over a period of three years.

Both nephritis and toxemias of pregnancy seem to be general diseases affecting the cardiorenal vascular system as a whole.

When the toxemia of pregnancy is classified by the same method which Volhard uses for nephritis, a marked difference in the end-results is seen and this difference allows the physician to make a more accurate prognosis, both as to the mortality among the mothers and as to the fate of the child in subsequent pregnancies.

TYPHOID FEVER: An Unusual Case of Typhoid Fever, Ross, K. C. Med. Jour. Australia, May 22, 1926, i, No. 21, p. 580.

A case clinically typhoid, and proved to be so at autopsy, in which positive agglutination tests could not be obtained during the disease. An agglutinable typhoid bacillus was obtained at autopsy.

HEPATIC CIRRHOSIS: The Glycemia Curve in Cirrhosis of the Liver, Puxeddu, E. Clin. Med. Ital., March-April, 1926, lvii, 174.

The author studied the glycemia curve throughout the day in five patients with atrophic cirrhosis of the liver and in two normal subjects as controls. In each of them he compared the glycemia curve with the glycosuria.

He found that in patients with cirrhosis of the liver and other liver diseases the glycemia during fasting is normal, oscillating around 1:1000. The glycemia curve shows marked oscillations in patients with cirrhosis, which are entirely lacking in the controls in whom the line runs more slowly and regularly. There is also in the cirrhotic patients a hypoglycemic period preceding the ascending part of the curve. The hyperglycemia curve in cirrhotic patients generally shows a sharp rise, while the descending phase is slower and does not reach the starting point until after three hours and a half or even more. The hyperglycemia test should be used systematically in testing the glyco-regulating function of the liver instead of the alimentary glycosuria test which is not reliable and is always influenced by numerous outside agents.

MALIGNANT ENDOCARDITIS: Apparent Mutation of Streptococci from Acute Malignant Endocarditis, Howell, K., and Beverley, D. Jour. Infect. Dis., July, 1926, xxxix, No. 1, 12.

From the blood of a case which was clinically one of acute malignant endocarditis there were isolated on three successive occasions two bacteriologically distinct organisms, Streptococcus hemolyticus and Streptococcus viridans. The two strains were morphologically different; the morphologic differences have remained constant over a period of two years. The sugar reactions, which were also different, have remained constant for the same period.

The hemolytic streptococcus strain seven months after isolation lost its hemolyzing quality and remained constantly thereafter anhemolytic. One constant mutation, therefore, occurred.

Brief animal passage indicated that the two strains were different. It was impossible by this experimental procedure to split off a viridans variant from the hemolytic streptococcus or its anhemolytic form, or the reverse. Transient variants occasionally occurred.

The immunologic reactions, although variable suggested that there was only a single strain, the hemolytic streptococcus. It is possible that the parasitic growth of two closely related bacterial strains may so alter their immunologic reactions that differentiation by such reactions becomes impossible.

Protection experiments with immune serums indicated that the two strains were different.

MALIGNANT ENDOCARDITIS, TRANSFUSION IN: Antibody Response After Immunotransfusion in Malignant Endocarditis, Howell, K. H., Portis, B., and Beverley, D. Jour. Infect. Dis., July, 1926, xxxix, No. 1, p. 11.

In a case of acute malignant endocarditis, both Streptococcus hemolyticus and Streptococcus viridans were isolated from the blood on three different occasions.

During a period of five months the patient received twelve transfusions of immune whole blood.

Before immunotransfusion was begun his blood serum contained no agglutinin for the streptococci isolated. The opsonic index was 0.57 and the complement-fixation reaction with streptococcus antigen was weakly positive.

The serum of the donors at the time of transfusion had an agglutinin titer of 1:10, 240 or 1:20, 480.

The agglutinin titer of the recipient's serum increased after each transfusion, reaching after the later ones a figure higher than that of the transfused blood.

The opsonic index of the recipient's blood serum also increased after each transfusion, as a rule running parallel with the agglutinin titer, and reaching values higher than those of the transfused blood.

Complement-fixing antibodies also increased, but appeared later than agglutinin and opsonin and fluctuated more irregularly.

The increase in specific antibodies of the recipient's blood, both as compared with its own original content and with that of the immune donors, is interpreted as evidence of the development of active as well as passive immunity as the result of immunotransfusion.

At variable periods, usually two to three weeks, after each transfusion the agglutinin and opsonin titers of the patient's blood dropped suddenly to low figures, reaching a maximum again usually within twelve days after the next transfusion.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*Biological Relations of Optically Isomeric Substances**

THIS small volume represents the last work of the great master whose sudden death occurred only shortly after he had read the galley proof of the book. To the volume is thus added a certain historical interest, for one cannot read these pages without subconsciously bearing in mind the work on isomeric compounds in which this great leader in pharmacology had been interested for many years before his untimely death.

The volume consists of eighty pages and represents the third Dohme Lecture delivered at the Johns Hopkins Medical School in 1925, under the auspices of the Dohme Lectureship, founded by Mrs. Charles E. Dohme in memory of her deceased husband.

The book is divided into five sections as follows: Introductory and Historical, Relation of Enzymes and Optically Active Bodies, Decomposition of Isomers in Living Tissues, Pharmacologic Action of Optical Isomers, General Aspects of the Pharmacologic Action and Influence of Configuration on Pharmacologic Action.

To those familiar with Cushny's work on the hyoscyamines, atropine, and the related tropeines, the dextro- and levorotatory adrenalins, etc., this volume will come as a fitting summary of one of the most important pieces of research contributed by the author. To Cushny's mind the varying reactions occurring (or not occurring) between dextro- and levorotatory compounds (either separately or in racemic form) and optically active elements occurring in the constitution of living tissues affords one of the best, and probably one of the most accessible, keys to the solution of the general problem of drug reactions. And one can read between the lines the unexpressed wish of this true scientist, that the work which he had so devotedly and hopefully pursued along these lines for many years might be carried forward vigorously and faithfully by other hands.

The book deals more with theoretical than with practical considerations. But to offset this the author, half apologetically, reminds us that the purely "theoretical" observation of the action of the pancreas on carbohydrate metabolism by V. Mering and Minkowski led to the introduction of insulin. And the casual observation of Brunton (while watching Gamgee study methemoglobin formation) that nitrites lower blood-pressure led to the introduction of these compounds into practical medicine. And perhaps even farther fetched was the observation of Liebreich who discovered the hypnotic action of chloral while he was engaged in a study of the decomposition of organic substances by the tissues of the body.

Symbionticism and the Origin of Species†

THIS little volume contains 171 pages and is made up of ten chapters whose headings are as follows: Introduction, History of Mitochondrial Research, The Bacterial Nature of Mitochondria, The Behavior of Mitochondria, Symbionticism, Microsymbiosis, An Analysis of Symbiont Reactions, Symbionticism and the Origin of Species, Symbionticism in Relation to Heredity and Development, and Symbionticism and Organic Evolution. There is also a nine page bibliography.

*Biological Relations of Optically Isomeric Substances. By Arthur R. Cushny, M.A., M.D., LL.D., F.R.S., late professor of Pharmacology and Materia Medica in the University of Edinburgh (Formerly in the Universities of Michigan and London). Cloth, $2.00; paper $1.00. The Williams and Wilkins Co., Baltimore, 1926.

†Symbionticism and the Origin of Species. By Evan E. Wallin, Professor of Anatomy, School of Medicine, University of Colorado. Price $3.00. Williams & Wilkins Co., Baltimore, 1927.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

The author states that the mitochondria, tiny bodies found in the cells of all living things, are themselves living organisms. The presence of such microbial invaders has been known, in many instances, to give rise to new tissue and organs. The mitochondria, therefore, may be the clue to unraveling the perplexing mystery of the origin of species.

Two points of view are held in regard to the nature of these microscopic mitochondrial structures, and this circumstance appears to be responsible for the diversity of opinion as to the activities of these bodies. The most commonly accepted view holds that mitochondria are cell-organs derived from the cytoplasm. The other view, that they are microorganisms "symbiotically" united to the cell, has attracted only a few adherents and apparently has been looked upon as a fantastic and improbable theory.

During the past seven years the author has investigated the nature of mitochondria, and has arrived at the unqualified conviction that these bodies in the cell are bacterial in nature. It has been evident that a large number of biologists have been skeptical of the results obtained in these investigations, and it also appears that many have been opposed to the fundamental conception that mitochondria are bacteria. It is the chief object of this book to summarize the evidence for the bacterial nature of mitochondria, and to present some of the evidence that demonstrates the fundamental rôle played by bacteria in the origin of species.

The book is interesting and stimulating and covers a wide range of biologic phenomena. Obviously it would be most unusual if all the hypotheses that are advanced in the book should in the end prove to be correct. But nevertheless they may serve as a splendid intellectual ferment to stimulate biologists, pathologists, and bacteriologists to further and more comprehensive researches. Incidentally the book serves to emphasize in a very striking manner the great divergence of present day "anatomic research" from that which was carried on in the average medical school of two or three generations ago.

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*A History of Medicine**

H. G. WELLS, in the introduction to *The Outline of History,* comments upon the unsatisfactory condition of the teaching of history as a part of a general education due, in part, to the lack of available time for a generalized study, and in part to a narrow and partial approach to this subject. Whether Wells has been successful in his attempt to treat history as a whole is neither here nor there for present purposes. His contention in the above paragraph, however, is difficult to deny in regard to history in general and cannot be disputed with regard to medical history.

It is a matter for thought, and even an occasion of surprise in these days of scientific and even ultrascientific medical education that so little time is given to, and so little interest aroused in the history of this, one of the greatest of the professions. It is surprising that only within very recent years have Chairs of Medical History been established in a few of the larger schools, and it is to be deplored that so little, and sometimes, indeed, no place is given to medical history in the curriculum of others. To paraphrase Wells, some of this neglect is undoubtedly due to the difficulty of presenting a satisfactory survey of the subject in a limited time, for it is manifestly impossible to consider medical history as the history of Hippocrates, plus the history of Galen, plus the history of Empedocles and so on, even though one had an influence upon the other and all upon the development of the medical art in general.

For the individual deeds of these worthies special texts should be consulted, but to appreciate the compelling interest of the evolution of medicine, a broad and comprehensive survey, a panoramic and philosophic consideration, is required as a starting point for interest leading to collateral reading.

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*A History of Medicine From the Time of the Pharaohs to the End of the Eighteenth Century. By C. G. Cumston, M.D., Lecturer on the History of Medicine in the University of Geneva, with an introductory essay by F. G. Crookshank, M.D. Cloth. Pp. 390. 24 Plates. Price $5.00. Alfred A. Knoof, New York.

Such a survey is the purpose of Dr. Cumston's book, which is one of a number of volumes, some written and some to be completed, comprising as a whole a history of civilization, whose purpose is to present a universal survey of the history of mankind.

The necessity for such a book as Dr. Cumston's and the justification, if any were demanded, for the labor, time, and scholarship expended upon its preparation is forcibly propounded in the introductory essay by Dr. Crookshank upon *The Relation of History and Philosophy to Medicine.*

Excellent as is the history, this essay is no less admirable, and if nothing else in the book were read, its purchase would have been profitable.

Its opening paragraph is as follows:

"Whilst the successful, nay, the competent practice of Medicine—an Art which includes that of Surgery—may be, and often is, compatible with ignorance of the History of Medicine, he is the best physician in the classical and fullest sense of the word who unites a mastery of his Art to an intimate acquaintance with the great historical doctrines and the philosophies upon which they are based."

With this as his thesis, Dr. Crookshank develops the thought that all too often the modern practitioner is but ill-equipped for intellectual expansion and, in a sense, anticipates what Cumston says in his introductory chapter, that "the abuse of erudition certainly need not be feared in medicine at the present time."

Dr. Cumston's book consists of thirty-one chapters in which the history of medicine is surveyed and recounted, not as a history of successive or selected individuals, but as an interlocking series of complementary personages and events, each with an influence not only on his own time but on ours and on the evolution and development of medicine as a whole.

The style is clear and crisp, interesting and readable, and amply reflects the scholarly ability of the author; it continually reminds us of Littre's dictum: "There is nothing in the most advanced contemporary medicine whose embryo cannot be found in the medicine of the past."

There are few who will read this book without interest and none can read it without profit. It is a book to buy, not to borrow.

The plates are all of historical interest and mainly from the author's collection. There is an excellent index.

In typography and make-up the book is an example of excellent craftsmanship.

The foreword quotes from Andrew Lang and with his words this comment can conclude: "The little present must not be allowed wholly to elbow the great past out of view."

*The Treatment of Chronic Deafness**

WHILE this small book of eighty-five pages has reached the dignity of publication by the Oxford University Press, the contents remind one of the instruction books furnished with various electrical treatment outfits. The instructions for the use of the "electrophonoide" are extremely minute.

Dr. Zund-Burguet, who designed the apparatus, is not a medical man but a doctor of science, and the apparatus itself was devised primarily to educate the deaf-mute.

Dr. Catheart became interested in it through its effect on his own disability and recounts the history of one hundred cases, including nerve deafness, chronic otitis media, otosclerosis, etc., in all ages., 68 per cent of whom were "definitely improved after treatment."

The author forestalls his critics who will say that the method is unscientific by reminding them that all science is founded on empiricism, and that certainly any treatment that holds out hope in chronic deafness deserves a trial.

*The Treatment of Chronic Deafness by the Electrophonoide Method of Zund-Burguet. By George C. Catheart, M.A., M.D. Pp. 85. Cloth. Price $1.35. Oxford University Press.

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EDITORIALS

The Pharmacologic Action of Carbon Monoxide

THE poet has well said that death has all seasons for its own, but there is one season in particular which carbon monoxide has for its own, and that is the winter season. Already numerous reports of deaths from carbon monoxide poisoning are appearing in the daily press, and this, too, in the face of almost daily admonition to avoid the dangers of this exceedingly widespread poison.

This substance is very largely a product of civilization, and its fatalities show that although human progress may in many ways thwart the destruction of human life, still there are other ways in which it may set the pace for death. In former times numerous fatalities occurred as the result of using charcoal in open heaters in tightly closed rooms in which the victims, often at the end of the day, easily passed into final sleep. But with improvement in modern heating facilities the old-time charcoal heater mainly fell by the wayside, and with the more extensive introduction of artificial gas for cooking and heating death by the carbon monoxide route became more and more an urban luxury. But with the advent of the modern automobile

the tide has again changed and carbon monoxide, with much accompanying stench, has now polluted the atmosphere of the uttermost parts of the earth. Indeed in many of those spots where the sky is still clear and the air is pure the future holds some promise that by and by man's flying propensities in the form of fast mail delivery, or of cigarette advertisements, or perhaps in the admonition to heed the virtues of certain political aspirants or the latest forms of hair restorers, may still further extend the influence of this deadly gas.

Clinically the cases have been divided into acute, chronic, and relapsing. And among the latter are to be found a variety of delayed neurologic phenomena often especially characterized by central or peripheral paralyses, speech disturbances, loss of memory, etc. The peculiar mental disturbances, the involvement of the lenticular nuclei, etc., in a way remind one of the old-time cases of severe chronic lead poisoning.

Since the publication of Claude Bernard's work it has almost universally been believed that carbon monoxide acts in the body solely by forming a firm combination with the hemoglobin of the red corpuscles. This results in preventing the corpuscles from carrying oxygen to the tissues and consequently the acute symptoms are practically identical with those of a slowly induced asphyxia with the exception of the color of the blood which is bright red as the result of carbon monoxide hemoglobin formation. This view has not passed entirely unchallenged, however, for Vahlen, Huebner, Geppert, Giacosa and others have from time to time pointed out certain features of the action of carbon monoxide, either when pure or when mixed with other substances as in illuminating gas, etc., which do not apparently exactly coincide with results which might be expected to be produced by asphyxia pure and simple.

Haldane[1] (J. S.) believed long ago that he had demonstrated completely that a mouse which was placed in pure oxygen under two atmospheres pressure, and whose blood would therefore absorb sufficient oxygen to render the animal independent of the specific respiratory action of the hemoglobin, could be treated with any amount of carbon monoxide without being poisoned thereby.

Recently, however, new ground has been broken in this field. Warburg[2] has found that when the proportion of CO to O_2 in a gas mixture was raised to about 5, the O_2 consumption of yeasts and a coccus was diminished. He also showed that strong light neutralized the effect of CO. He concluded that CO combined with a *catalyst in the cells* with which O_2 must combine before it can oxidize other substances, and that this catalyst was probably an iron compound analogous to hemoglobin. As the amount of CO needed to cause a given drop in the O_2 consumption was roughly proportional to the partial pressure of the O_2, the analogy with hemoglobin could be carried still further. And in line with this suggestion Haldane[3] (J. B. S.) has extended this work and added new evidence to that already accumulated. Haldane has worked with wax moths (Galleria mellonella), with the seeds of cress (Lepidium sativum), and with rats, thus extending the observations to a mammalian form and thereby involving the action of CO in the presence of hemoglobin.

Haldane subjected these animals to varying percentages and pressures of CO and O_2. The actions of the moths under very low but progressively increasing percentages of oxygen were compared with their movements with addition of CO under similar conditions. The germination (splitting) of the seeds were similarly studied. The rats were placed in an air-tight chamber and subjected to pressures of one or more atmospheres of either pure oxygen or of oxygen and carbon monoxide together. When a rat was placed in three atmospheres of oxygen and one of carbon monoxide, its behavior was almost normal, but in some cases the movements of the hind limbs were rather clumsy. In two atmospheres of O_2 and one or one-half an atmosphere of CO, movement was difficult, but there was little increase of breathing and no convulsions. It was shown by J. S. Haldane[4] (1912) that at three atmospheres of O_2 and one of CO the rat's hemoglobin was about 98.3 per cent saturated with CO, the remaining 1.7 per cent being a very inefficient carrier of O_2. Since a single atmosphere of CO is sufficient to nullify practically all the hemoglobin, the effects of any further addition of that gas can only be on the tissues, since the addition of a physiologically inert gas, such as N_2, is without effect. The rats used in these latest experiments (J. B. S. Haldane) showed very different susceptibilities to high pressures of CO, all from the same cylinder. Thus while Rat H died on exposure to 3 atmospheres of O_2 and 2 of CO, and Rat F in 2 of O_2 and 2 of CO, Rat B appeared practically normal after four minutes in this mixture, though it died on raising the CO pressure to 3 atmospheres. And Rat D appeared little affected by four minutes exposure to 2 atmospheres of O_2 and 3 of CO, though it was killed by an additional atmosphere of CO. Haldane suggests that these differences may be due to different relative affinities of the catalyst for CO and O_2, but that it seems equally plausible to trace them to differences in the cerebral vasoconstriction caused by O_2 (Tinel, 1927).[5] No definite evidence was obtained that poisoning by CO at high pressure can be relieved by high pressures of O_2, but attempts to prove this were rendered difficult by the fact that convulsions due to acute O_2 poisoning set in at about 7 atmospheres pressure of O_2. It was, however, clear that CO has a poisonous action on rats apart from its combination with hemoglobin. It is very unlikely that the catalyst, or substance sensitive to CO, is a mere carrier of molecular oxygen like hemoglobin. The nature of the catalyst is, of course, unknown, though it may well be an iron-porphyrin derivative. Oxygen activated by the catalyst in question may be required for the oxidation of a variety of substances. As such catalysts have now been found in bacteria, yeasts, higher plants, insects and mammals, they are presumably present in the majority, if not all, aerobic organisms.

Haldane also brings out one other point of interest and that is that all his samples of CO had a definite smell, generally compared to that of garlic or tar, and this was not changed by thorough purification. It is therefore likely that odor is a physiologic property of CO. It is only observed in fairly high concentrations, which may of course, be smelled with safety provided the operation is not repeated too often at short intervals. But the chief conclusion to be drawn from Haldane's work is that rats living on

oxygen dissolved in their blood in the presence of sufficient CO to combine with almost all their hemoglobin are killed by the addition of more CO, which must affect some substance (a catalyst) in their tissues.

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—*D. E. J.*

Oskar Minkowski

THE thirteenth of January, 1928, will mark the seventieth anniversary of the birth of Oskar Minkowski, and we desire to take the opportunity to place on record our high regard for the contributions of this great master in medical research. We beg to offer him our congratulations and our good wishes for a happy retirement.

Minkowski ranks as one of the leaders in the investigation of the problems of metabolism by experimental methods, and his paper on pancreatic diabetes published in 1893 (Arch. f. Exper. Path. u. Pharmakol., 1893, xxxi, 167) is justly considered by those who are familiar with it to be an example of what such papers should be.

Born in Kovno in West Russia, Minkowski moved to Königsberg when he was thirteen years of age. Here he studied medicine and after receiving the M.D. degree, he became attached to the clinic at Naunyn, who was then at Königsberg. Shortly afterward Naunyn moved to Strasbourg, and Minkowski accompanied him, being greatly interested in the chemical and experimental investigation of disease. Naunyn established in Strasbourg a laboratory of experimental medicine in connection with his clinic, this being the forerunner of the numerous laboratories of this type now to be found both in Europe and in this country. Realizing the importance of a thorough knowledge of organic chemistry for research in this field, Minkowski also worked in the pharmacologic laboratory of Schmiedeberg. About this time the idea of acidosis was forming itself in the minds of men, mainly through the work of Walther and Stadelman, and Minkowski discovered that in diabetes such a condition was caused by the presence of oxybutyric acid in the urine. Minkowski was greatly interested in these discoveries, and it was about this time, 1889, that von Mehring, who was professor of State medicine in the University, a former disciple of Frerichs, and the discoverer of phlorizin diabetes, consulted him with regard to the possibility that a fat, which he called lipanin, might be absorbed from the intestine without being digested. To test this possibility, Minkowski proposed feeding the fat to animals (dogs) from which the pancreas had been removed. In an animal in which this was done, it was noticed that the dog was passing urine with great frequency. This suggested the possibility that diabetes might be present, which was soon confirmed by obtaining a positive test for sugar in some of the urine which

had been voided on the floor. The significance of this discovery was immediately grasped by the two investigators, and for the next few years Minkowski devoted himself to a thorough investigation of pancreatic diabetes, finally publishing his results in the paper above referred to.

Since the publication of that paper Minkowski has at various times contributed further facts with regard to experimental diabetes, and indeed has so completely covered the field that little has been left for others to add. Among other things he established the significance of the D:N ratio and of the almost complete recovery in the excreta of ingested sugar. He also showed that the diabetes was not due to the absence of the digestive secretion of the pancreas, since it did not occur when a graft was left in the abdominal wall after removal of the main gland, and he suggested that the absence of some secretion normally produced by the pancreas might be the responsible cause, failing at first, however, to accept the hypothesis of Laguesse that this internal secretion was produced in the isles of Langerhans. He tried the effect of administering extracts of pancreas to diabetic dogs, and although this part of his work was not crowned with success, his discovery of pancreatic diabetes must be regarded as an absolutely essential step, leading to the demonstration and isolation of insulin.

Besides working in the field of diabetes, Minkowski has contributed many other important facts in animal metabolism; for example, the origin of uric acid. It was in connection with this work that he discovered that the liver could be removed from geese without interfering with the circulation, on account of the natural anastomosis between the portal vein and the vena cava which exists in these animals. After removal of the liver, he found that uric acid became replaced in the urine by urea and ammonium lactate.

It is of interest to note that a brother of Minkowski, Hermann, was one of the foremost mathematicians of his day, being one of the teachers of Einstein and contributing, just prior to his premature death, a most important contribution to Einstein's theory. —*J. J. R. M.*